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THE PERSONALITY, HEREDITY AND WORK OF CHARLES OTIS WHITMAN, 1843–1910

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COLD SPRING HARBOR

AT Pinhook, in the town of Woodstock, Maine, was born December 12, 1843,[1] a male child named by his parents, Joseph and Marcia Whitman, *Charles Otis*. Some time thereafter the parents and their children removed to Waterford (where they were in the '50s) and in 1861 to Bryant's Pond (Fig. 1). All of these places are in the southern part of Oxford County, 50 miles or so from the coast at Portland, in about the latitude of the Presidential Range of the White Mountains—a country of high hills and small mountains, covered with pine and hardwood forests, abounding in lakes with some fertile plains between; a country affording a stimulating environment to a boy with an inherent love of nature. And a love of nature was widespread in the boys of this country and in their fathers. Take, for example, Jacob Whitman. Born in Easton, Mass., twenty miles south of Boston and ten miles north of Taunton it would appear that his lines had been pleasantly cast. But[2] he had an adventurous spirit and was perhaps "very wilful." He was with the "min-

[1] The date is given as December 14, 1842, by Lillie, 1911, and Morse, 1912; also National Cyclopedia of American Biography, XI, p. 73. The date given above is that of the Whitman Genealogy. It also agrees with that remembered by Mrs. H. D. Smith (Mary Whitman) because her sister Sarah was born September 10, 1843, and Charles O. was born the December following.

[2] Cole and Whitman, 1915, p. 42.

ute men'' in 1775, probably fought at Bunker Hill, was at Harlem Heights, stormed Stony Point, helped capture Saratoga, and was at Trenton. He had married, 1777, and, after the war, 1781, came to the frontier settlement of Buckfield, Maine, which had been settled some four years before. Fellow townsmen, by the name of Record,

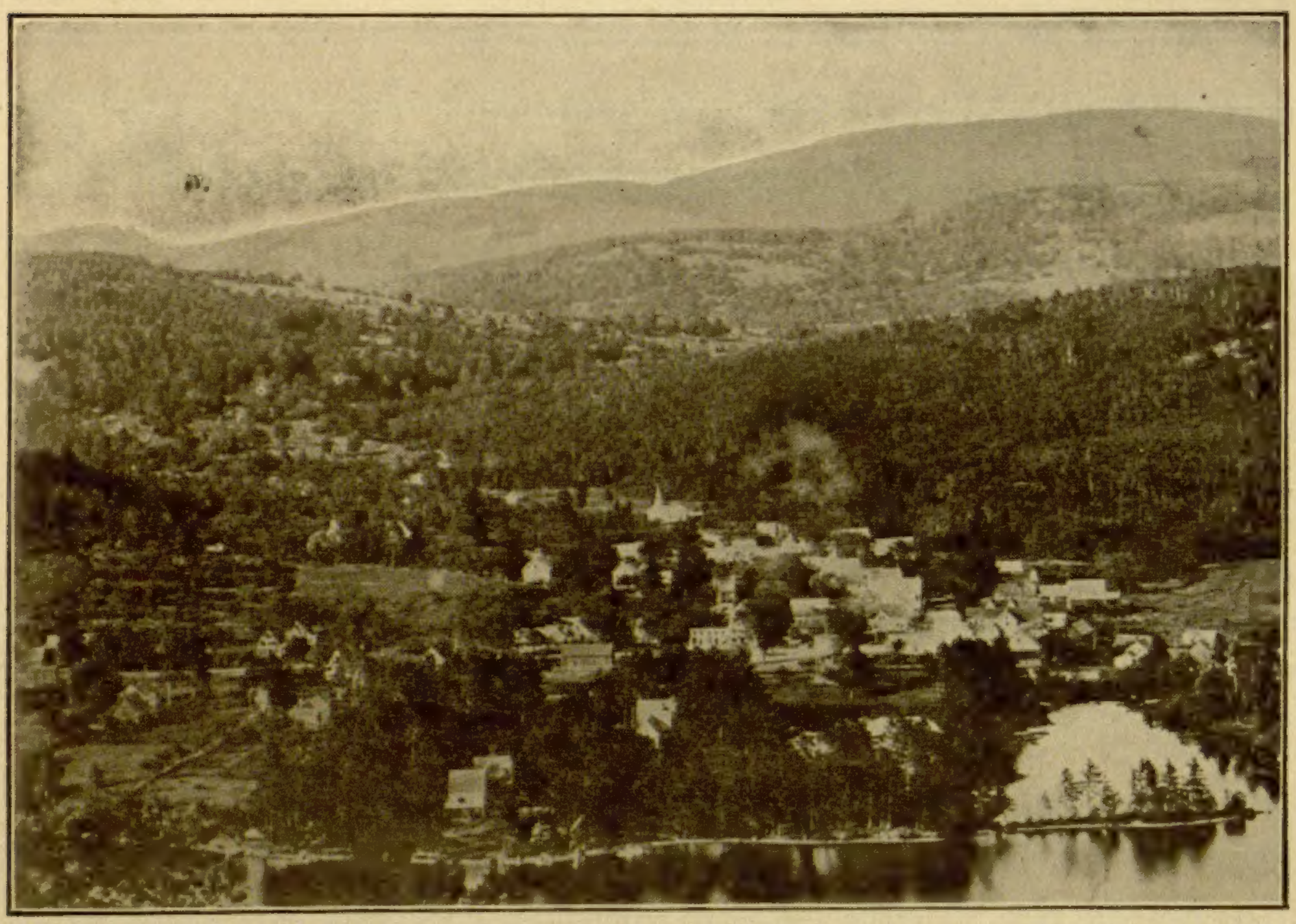

FIG. 1. View of village of Bryant Pond, Woodstock, Maine, home of Charles O. Whitman during early manhood. Note the church in the center, the school house with a bell tower to the left of the church and more toward the foreground. The Whitman homestead lies to the right of the school house, partly screened by trees. The pond is in the foreground and is backed, as seen from the village, by a lofty cliff from which the photograph was taken. Over these hills and through the pine and hardwood forests the young naturalist, Whitman, hunted. (Contributed by Professor Whitman's sister, Mrs. E. B. Reynolds, of Lynn, Mass.)

had previously settled there and had told Jacob Whitman about the town and their tales of conditions at Buckfield evidently appealed to him, and induced him to migrate thither.

> He was a stout and muscularly built man . . . and was inured to hardship. . . . He was an industrious citizen and paid strict attention to the clearing up of his lot, rearing a family and attaining a competence. He was often selected for road surveyor, school agent, and school committee and his name was always kept in the jury box until he got too old to attend to such service. He was a man of fierce spirit when aroused and positive in his opinions and fond of argument.

He declined to join the first church started at Buckfield as he did not believe its creed. Of other founders and early settlers of Buckfield it is known that they were hunters and were attracted to the locality by observations made on hunting expeditions. Such were Nathaniel Buck "a man of great physical strength and endurance, and noted for being an expert hunter and skilled in woodcraft"; Thomas Allen, a deserter from the English army to the side of the colonists, a man of adventurous disposition, fiery temper and obstinate in his opinions; and Benjamin Spaulding who had retired from Chelmsford, near Lowell, Mass., to this wilderness, partly to avoid certain financial obligations and partly to trap and hunt.[3] Oxford County was, indeed, as much a frontier of civilization at the end of the eighteenth century as the Rocky Mountains were fifty years later and attracted much the same sort of adventurers, lovers of untouched nature, the forests and wild animals. It was the sort of blood from which naturalists might be expected to arise, and from Oxford County and from the adjacent counties of Cumberland and York have arisen such men as A. E. Verrill, of Greenwood, H. C. Bumpus, of Buckfield, and C. O. Whitman, of Woodstock (Oxford Co.); E. S. Morse, of Portland; A. S. Packard, of Brunswick (Cumberland Co.); Geo. B. Emerson, of Wells, and Geo. L. Goodale, of Saco (York Co.); and Elliot Coues from just over the boundary at Portsmouth, N. H.

Charles Otis Whitman was born December 12, 1843, in Woodstock, Me. As a boy he attended the town schools at Woodstock and Waterford, and worked on the home place. During the summers of 1857 and 1858, while at Waterford, he helped his father's brother, Elhanan, on the farm, as Elhanan's daughter, Mrs. H. D. Smith, of Norway, Maine, recalls very well. He was a good-natured boy and good company to his cousins. His cousin can not recall that he was engrossed in birds at that time; but by 1860 he had made a considerable collection which

[3] Cole and Whitman, 1915, pp. 24–5.

FIG. 2

FIG. 3

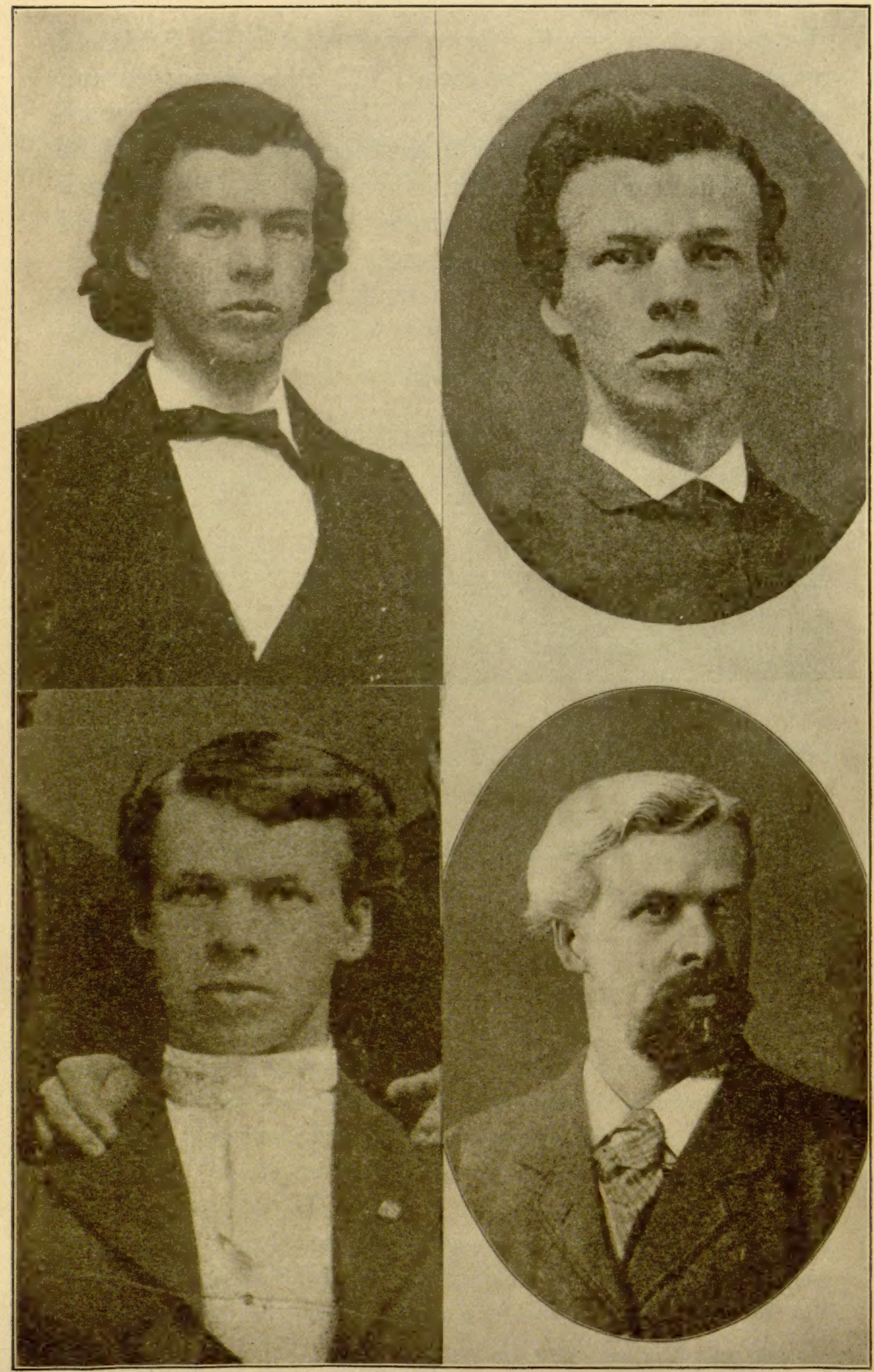

FIG. 4

FIG. 5

FIG. 2. Charles O. Whitman, at the age of eighteen years. This picture shows a great resemblance to his son, Carroll Whitman, at the time he entered Cornell University. From a tintype contributed by Mrs. E. B. Reynolds (Myra Whitman).

FIG. 3. Charles O. Whitman at the age of about twenty-one years. Reproduced from Lillie, 1911; where it is labeled, "1857, enlargement from a tintype. Kindness of Professor E. B. Morse." This would place the age at fourteen years. His cousin Mrs. H. D. Smith assures me that the correct age is as stated.

FIG. 4. Charles O. Whitman at the age of twenty-five years. This is one of a group and is probably of his class at graduation from Bowdoin College. (Note the "class pin.") Some of the other members of the group are full-bearded young men. From a silver print contributed by his sister, Adrianna Whitman Evans of Lynn.

FIG. 5. Charles O. Whitman at about thirty-nine years.

Fig. 6

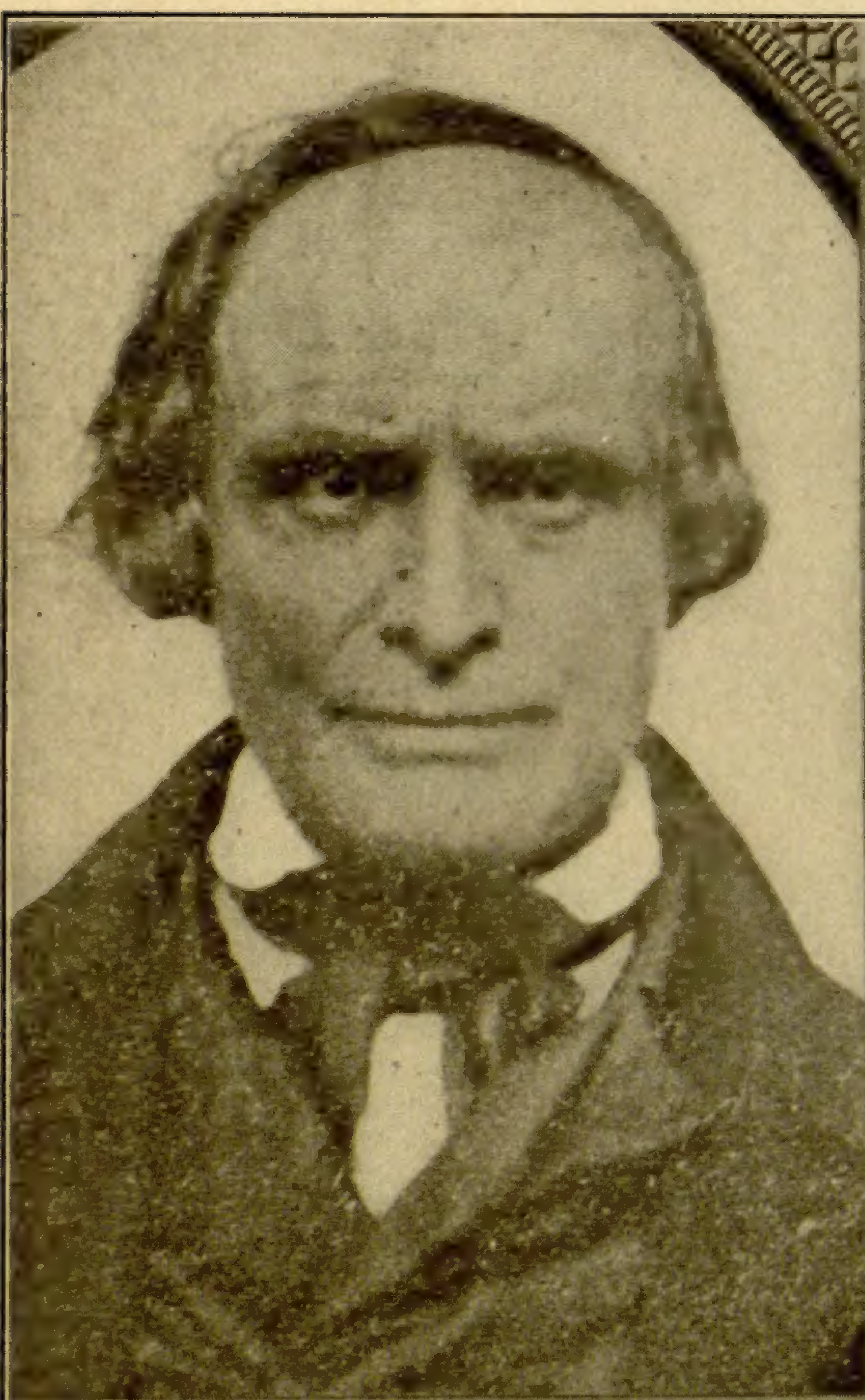

Fig. 7

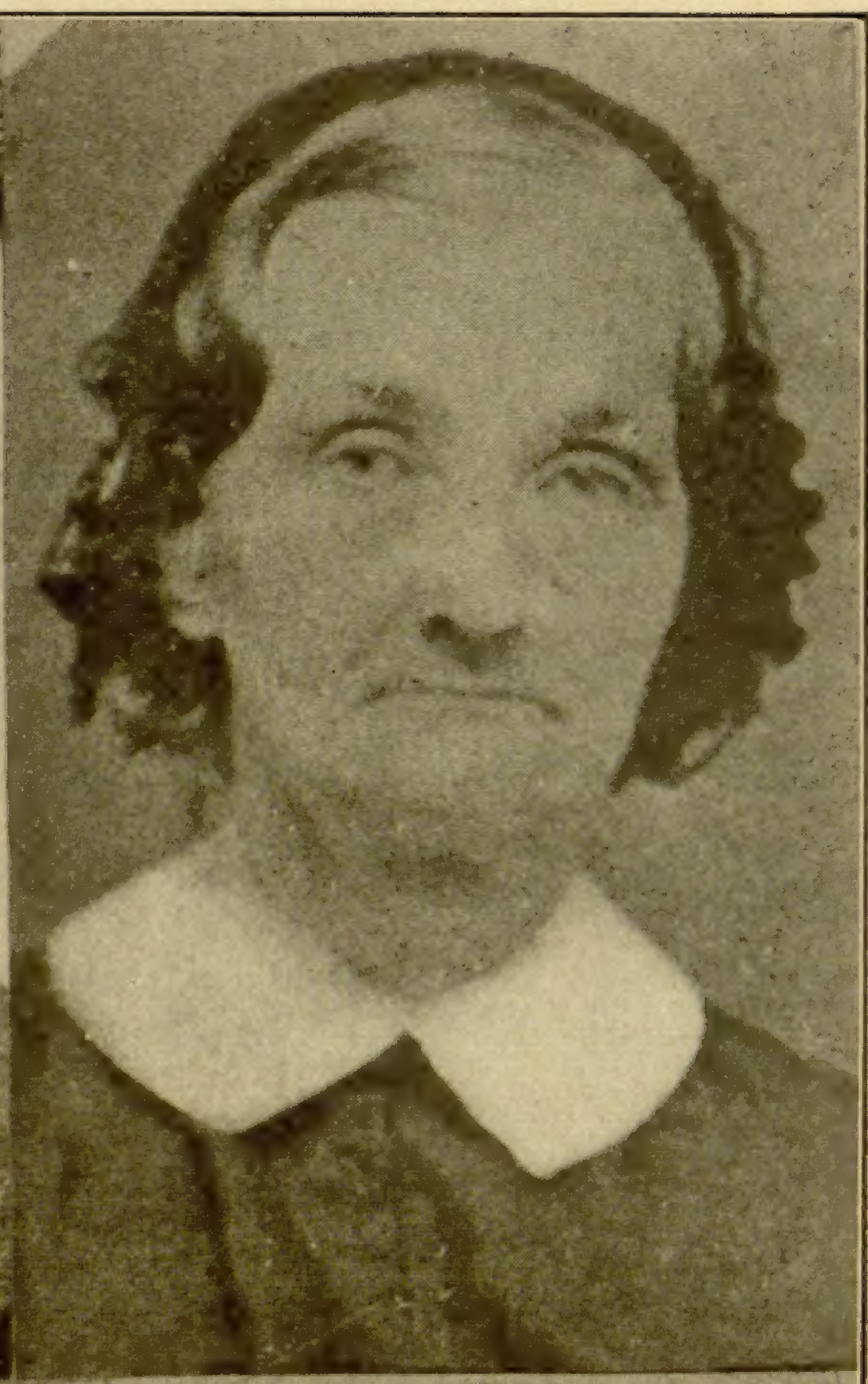

Fig. 8

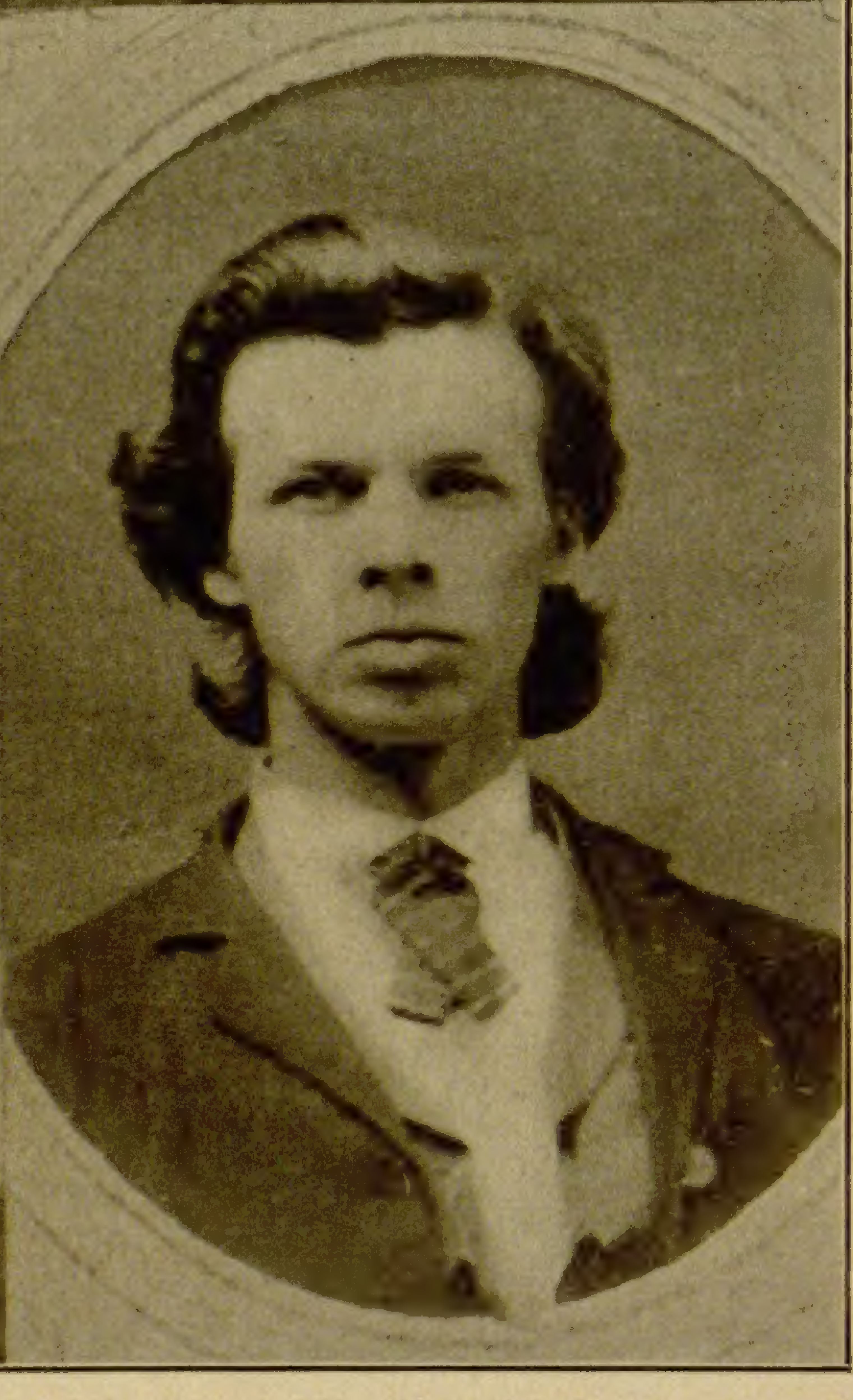

Fig. 9

FIG. 6. Solomon Leonard, the father of C. O. Whitman's mother, from a daguerreotype in the possession of Mrs. Myra Whitman Reynolds.

FIG. 7. Esther French, wife of Solomon Leonard and mother of C. O. Whitman's mother. From a daguerreotype in the possession of Mrs. Reynolds.

FIG. 8. Marcia Leonard, wife of Joseph Whitman and mother of C. O. Whitman. Note the thick lower lip and slightly raised tip of nose. From a photographic print; kindness of Mrs. Reynolds.

FIG. 9. Charles O. Whitman, at about twenty years, for comparison with his mother. Note the restricted broad lip and the slightly raised tip of nose. From a tintype; kindness of Mrs. Smith.

FIG. 10

FIG. 11

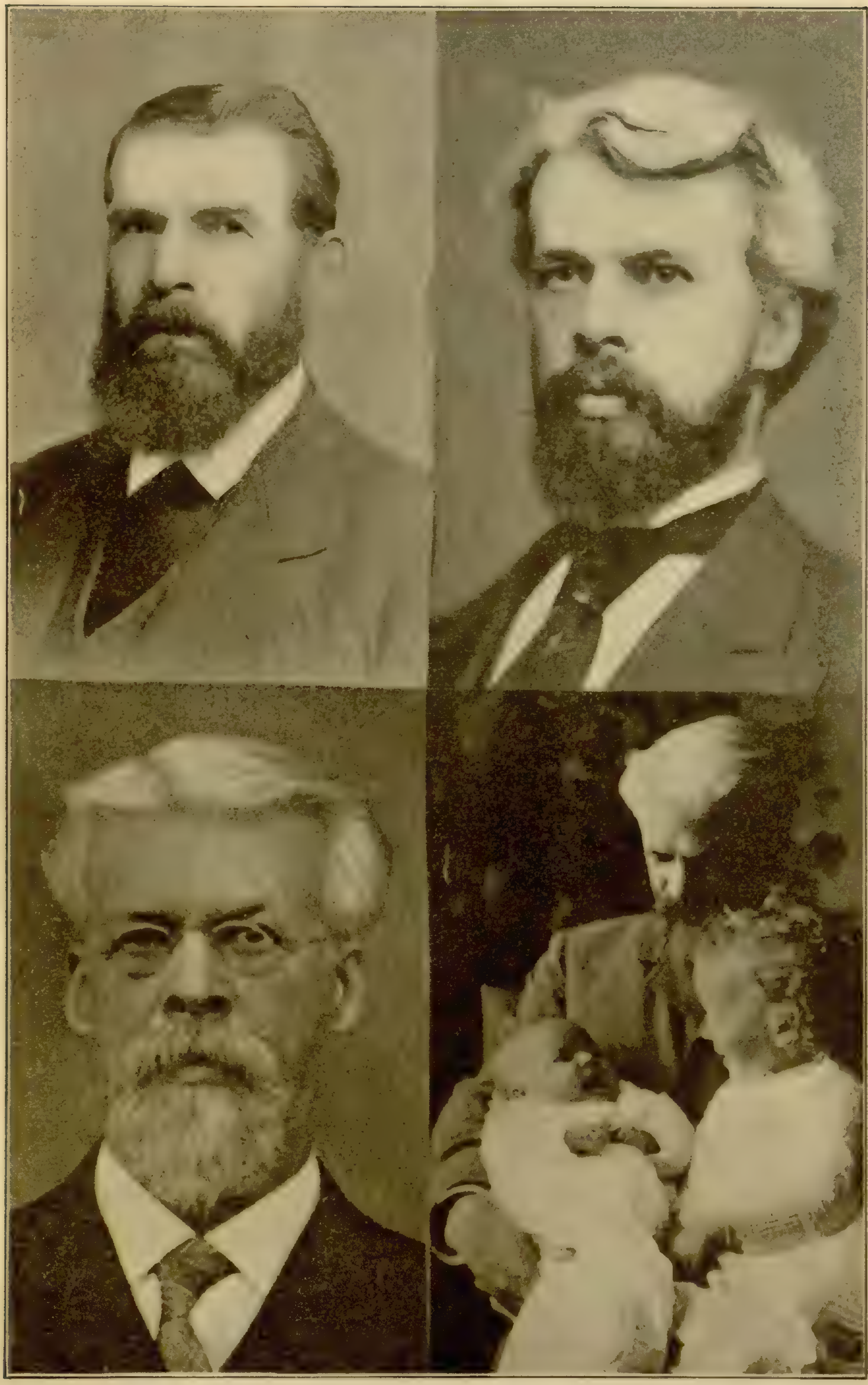

FIG. 12

FIG. 13

FIG. 10. Joseph Whitman (2d), father of Charles O. Whitman. Note the wide nostrils, long tip of nose and wavy hair, turning gray.

FIG. 11. Charles O. Whitman, probably on his return from Germany, about 1878, age thirty-five years. The hair of the head is now quite gray.

FIG. 12. Charles O. Whitman, professor at University of Chicago, probably spring of 1900. Age 56.

FIG. 13. "The Father," about 1887.

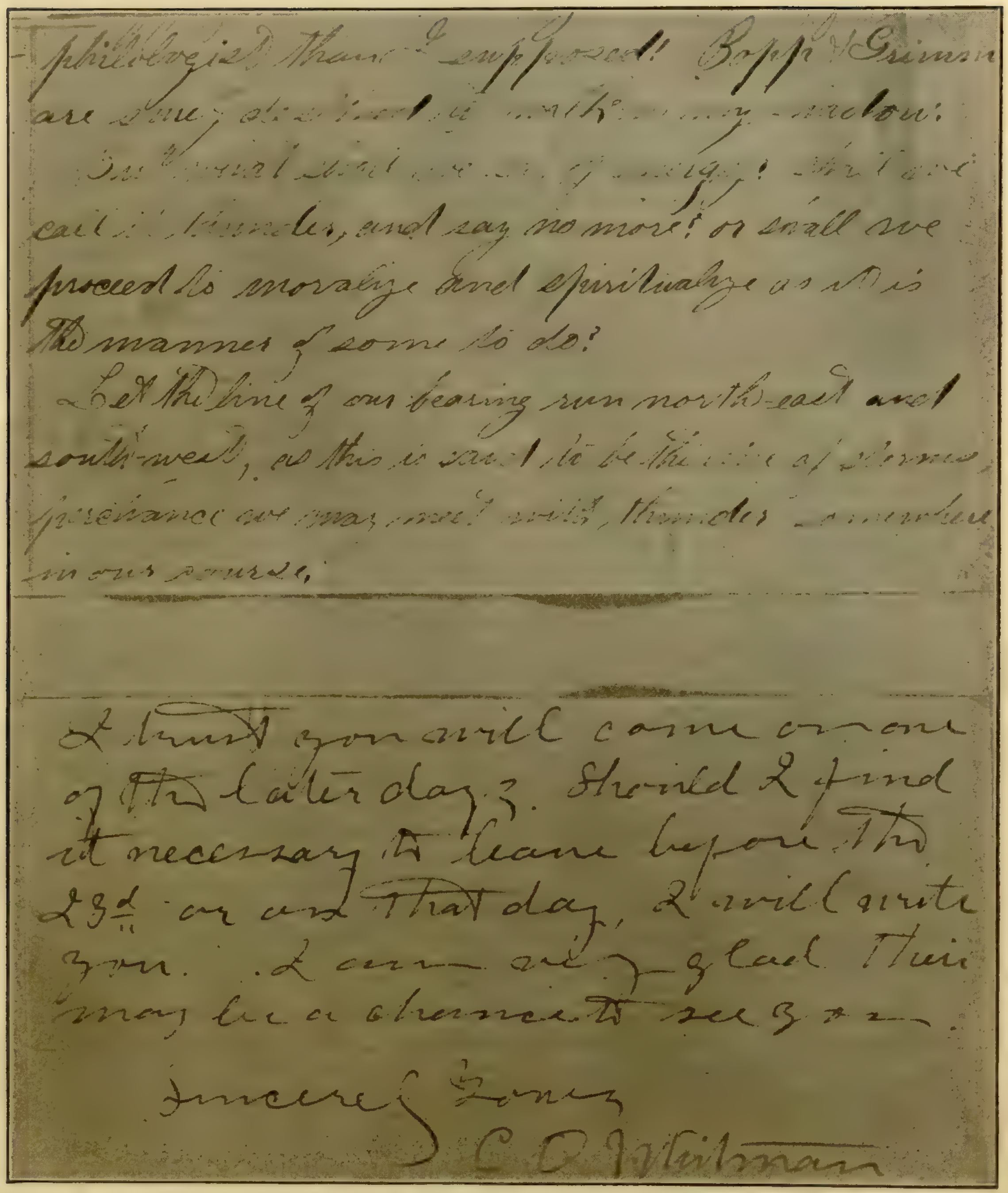

FIG. 14. Handwriting of C. O. Whitman. Above, at Westbury Academy at about the age of twenty-six years. Below, at fifty-six years.

he had mounted in two great glass cases, nearly covering one side of the room. Lapham (1882, p. 166) says:

> So artistically prepared were they, and so naturally mounted, that they attracted much attention among ornithological students.

It is said that he was dissuaded from volunteering for the Civil War by his father's opposition; was drafted but re-

jected after examination. In the autumns of 1861, '62 and '63 and in the spring of 1864 Charles attended the Norway "Liberal Institute," an academy of high school grade, where his uncle, George F. Leonard, taught. It is said that he taught winters to obtain the means for paying his school expenses.

He entered Bowdoin College as a sophomore in September, 1865, and was given the degree of B.A. in July, 1868. Lillie attributes Whitman's strong views in favor of the requirement of Latin for a college education to the emphasis laid in his day on the classics at Bowdoin. He participated in the student literary and debating interests. He ranked about ninth in a class of 23, his rank having been decreased through the necessity he was under of teaching to earn funds for his college expenses. The following autumn he was appointed principal of the Academy at Westford, Massachusetts, and continued in this capacity until the spring of 1872. Here he apparently taught a variety of subjects. In September, 1872, he was appointed sub-master at the English High School in Boston where his uncle, George F. Leonard, had been for some years master. Here he taught general high school subjects until the summer of 1875. Morse states that he distinguished himself there by his original methods in teaching certain branches of elementary science.

"While in Boston," says Lillie, "Whitman came under the influence of Louis Agassiz and was one of the 50 students who, in July and August, 1873, attended the Anderson School of Natural History founded by Agassiz on the island of Penikese." Here he met Professor E. S. Morse, one of the instructors, who became interested in him because of his drawings, superior even to Morse's own. Whitman returned to Penikese in the summer of 1874, after which the laboratory was abandoned. The short-lived Penikese laboratory greatly impressed those who attended it and several of these became founders of other marine laboratories—thus, Hyatt of a laboratory at Annisquam, Whitman of one at Woods Hole, Franklin W. Hooper of one at Cold Spring Harbor, David Starr Jor-

dan of one on the Pacific coast at Pacific Grove, California. It was at Penikese, says Morse, that Whitman seriously began his life work in science, for shortly afterwards he went to Dr. Dohrn's laboratory at Naples and then to Leipzig where, under the great Leuckart, he learned methods of microtome section-cutting, staining of tissues, and processes of preparation far ahead of those used at Penikese; in short the modern methods of the embryologist and morphologist." He was in Germany for three years, being on leave of absence all this time from the Boston high school. In 1878, at the mature age of thirty-five, he received the degree of doctor of philosophy from the University of Leipzig and in the same year appeared his doctor's thesis, "The Embryology of Clepsine," in the *Quarterly Journal of Microscopical Science.* This paper of 100 pages introduced important new principles, as well as facts, into embryological science and was beautifully illustrated by his own drawings.

On his return to America Whitman took up again his work at the Boston high school, teaching English; but at the end of the year he had decided to become a zoologist, applied for and received a fellowship in biology at Johns Hopkins University and resigned from the English high school. But during the summer of 1879 he received an invitation from Professor Morse, who had been teaching zoology at the Imperial University in Japan, to fill his place. Accordingly Whitman set sail for Yokohama, August 21, 1879, and taught at Tokyo until the summer of 1881. Here he trained four investigators who all became professors of zoology in the university: consequently he may properly be called the father of zoology in Japan. He and the administration of the university became estranged, as he would not adapt himself to what seemed to him a desire for official control of intellectual property; also he felt that his work was insufficiently supported. As he left he published a somewhat harsh brochure entitled "Zoology in the University of Tokyo." Leaving Japan in August, 1881, Whitman worked at the Zoological Station of Naples as a guest of Professor

Dohrn, November, 1881, to May, 1882. He was interested in, and recorded, the methods of microscopical research at that station, and published an important paper on the embryology, life-history and classification of Dicyemids, which had some time earlier but, as Whitman proved, without warrant been elevated to the position of a subkingdom of animals, the Mesozoa.

Returning to America in the autumn of 1882 he was appointed assistant in zoology at the museum of comparative zoology of Harvard University. While here he worked in cooperation with Alexander Agassiz on the development of pelagic fish eggs. Two papers were published on this matter and plates prepared, but no text, for another. At this time he published his book on "Methods of Research in Microscopical Anatomy and Embryology."

In 1886 Whitman was invited to take charge of a private laboratory for biological and related research that Edward Phelps Allis, Jr., had planned to found on the Lake at Milwaukee, Wisconsin. With Allis's cooperation Whitman then started the *Journal of Morphology,* the first of its kind in America and based on such high ideals of quality and of beauty of plates that the eighteen volumes published entailed considerable financial loss, so that finally it had to be discontinued—although it was revived, after a lapse of five years, under other auspices. The three years, '86–'89, at the Lake Laboratory saw the beginning of some researches by Whitman, which were mostly never completed, and by his associates, Howard Ayers, William Patten, A. C. Eyeleshymer, as well by the founder of the laboratory. Some of the published work is of the finest quality.

In 1888 the Marine Biological Laboratory at Woods Hole was organized by certain residents of Boston and Professor Whitman accepted their invitation to become its director. It was in the development of this laboratory that Whitman's greatest work for science was done. For he introduced and upheld ideals of cooperation and scientific democracy which led to its loyal and devoted support by a large body of the working biologists of the country.

In the year 1897 a disagreement occurred between Whitman and a minor, but influential, portion of the trustees. The latter regarded him as extravagant, while he thought they hampered the proper development and organization of the laboratory. Some of the Boston supporters of the laboratory thereupon withdrew from the board; however, the development of the laboratory suffered no serious interruption. At the end of twenty-one years as director Whitman was led to resign, owing to the growing demands upon his time of his researches.

It was in the early years of the Marine Biological Laboratory that Whitman and some of his co-workers saw the need of a technical zoological society and a call for such a society was made by a committee of which he was chairman. He served as president for the first four years. At first known as the American Morphological Society, it has been known since 1902 as "The American Society of Zoologists," and is still the national society for this science.

In 1889, Clark University having been organized exclusively for graduate and research work, Whitman was called from Milwaukee to become its professor of zoology. He now took up again his work as a teacher which continued to his death. There gathered about him at Worcester, Massachusetts, a small body of devoted zoological investigators. What with the *Journal of Morphology*, the Marine Laboratory and the organization of the new department his time was pretty well filled. He published three short papers on the leech, *Clepsine*. But affairs at Clark were not altogether to his liking and when a call to the University of Chicago came in 1892 Whitman and the heads of the departments of physics, chemistry, anatomy, neurology and paleontology seceded from Clark University and formed the major portion of the scientific faculty of the new university. Here at Chicago Whitman had more graduate students than ever before, but as his research grew more engrossing he kept more and more to his home where his experiments were conducted. For a period of fifteen years he bred pigeons to get at an under-

standing of the evolution of their color markings. He usually had about 500 individuals, representing about 40 wild species, in dovecotes surrounding his house. He hybridized nearly 40 species, most of which had never been crossed before. His work included the phylogeny of pigeons, instinct and animal behavior, voice, fertility and the nature of sex.

Professor Whitman suffered considerably from indigestion for some years before his death. He contracted a heavy cold while caring for his pigeons and died suddenly, December 6, 1910, at the age of 67, of pneumonia, the same disease that his mother died of at the age of 57.

We have now to consider some of the personal characteristics of Charles O. Whitman, and their distribution in his family.

First, white hair. Charles Whitman's hair turned white unusually early. Morse quotes Judge Clarence Hale of Portland as saying that his hair was perfectly white when in college but his class portrait (Fig. 4) shows his hair still dark. At the age of 39 (Fig. 5) the hair is quite white. His father's hair turned gray early; Charles's brother at 49 has very gray hair. Early graying is apparently a "Whitman trait."

2. Wave in hair is shown by his father (Fig. 10) and as a dominant trait is doubtless inherited from this side.

3. Nostril. His father had broad nostrils (Fig. 10), but the ridge is carried apically beyond the level of the nostril. In Charles, as in his mother (Fig. 8), the apical knob was lacking, hence the nostrils appeared unusually full.

4. The lower lip is thick, especially near the median plane. His mother's portrait (Fig. 8) shows an exceptionally broad lower lip and this is exactly reproduced in her daughter, Adrianna. The mother's father (Fig. 6) also has a broad lower lip. Charles gets the breadth of lower lip from his mother's side and its restriction to the middle of this lip (Fig. 9) is doubtless due to another factor.

5. The forehead is broad and full (Fig. 12); doubtless a

Whitman trait, as it is strikingly reproduced in his cousin, Mrs. Smith. Moreover his grandfather Leonard had an exceptionally fine head (Fig. 7).

6. The general slenderness of form is a Leonard trait. His father and some of his sibs were stout, as is one of his sisters, while his other sibs are slender, like himself.

Taste for Natural History.—This was, doubtless, inate and it was very strong, developed very early, as we have seen above, and led to his keeping animals and mounting birds. Thus he kept, as a small boy, mud turtles, rabbits, guinea pigs, woodchucks, and raccoons. Later, his sister recalls, he kept two gray squirrels which were so tame that they would go to sleep in his pockets. He kept doves, which he would tend carefully and of which he would watch the young after they were hatched. These doves would cover him as he fed them. He was fond of hunting. His stuffed eagle was especially famous. On one occasion, at the age of twenty-four years, he was gone two nights and a day after an owl. Mr. H. T. Libbey, of Bryant's Pond, told me that on one occasion he went out with Charles after a large bird and was gone all day. It is interesting that the special objects of interest of his early years should have been those of his late years. If we seek for the origin of this strong taste in such an out-of-the-way part of the world we find it, as stated above, in the blood of the pioneer, adventurer, hunter and lover of nature which sought the wilderness of Maine at the end of the eighteenth century. Actually, one finds evidence of love of hunting and of nature in close relatives, especially on the father's side. First, Charles's sister, Adrianna, was as fond of natural history as himself. She had a tame bluejay which stayed about the house like a crow. Charles's uncle, Chauncey C. Whitman, was a farmer who devoted himself, in his later years, particularly to the *raising of horses and cattle.* It is told of him[4] that, when he was twenty-four, he joined his fellow townsmen on a bear hunt and finally the bear was found in his den under the roots of a large tree. Chauncey "had the temerity to

[4] Lapham, 1882.

thrust in his arm and catch hold of her hair, when she quickly turned and came out before them all." She had two cubs. One was *taken out alive and kept for several months* by Chauncey Whitman until, having become mischievous, it was killed. Here we see the same love of wild animals that Charles showed. This Chauncey had a son, Albert, who is a hunter living in Oxford County. He keeps squirrels, woodchucks, and other pets. Albert's brother, Thomas, had a *poultry farm* and his son still carries on the business. Charles's father's father, Joseph Whitman, father of Chauncey, was also a hunter and told his grandchildren hunting stories, including adventures with bears and other animals, which are keenly remembered to this day. It appears that the mother's father, Solomon Leonard, had a love of nature and took pleasure in his trout brook. So Charles got his love of nature from both sides of the house.

Scholarship.—But Charles had more than a love of nature; he desired to *study* nature. This scholarship is a family trait and comes to him from both sides of the house. It is noteworthy that in Lapham's "History of Woodstock, Me.," 1882, p. 56, it is stated:

> Three Woodstock men have graduated from college, George F. Leonard, Harrison S. Whitman and Charles O. Whitman.

The first is an uncle and the second a cousin of the third. According to Cole and Whitman (1915, p. 707):

> Judge Mitchell's "History of Bridgewater" says that more persons of this (Whitman) name in that town in early times received a college education than any other. The next most numerous were Packards.

And Charles's father's mother was Abigail Packard. It is worth while to consider in detail the direction taken by the scholarship of the close relatives of Charles. First, the mother's father, Solomon Leonard, was a close student of ancient history. But it was to the influence of his mother's brother, George F. Leonard, that the direction of Charles's early life was chiefly due. George Leonard graduated from Dartmouth with the class of 1859.

> He adopted teaching as his profession and instructed in the academies in Norway and Paris, in Maine, and in Northbridge, Mass. He was

also a teacher in the English high school in Boston, following this occupation for over twenty years. . . . He is a profound scholar and succeeded well in his teaching.

It will be noted that Leonard was teaching at the English high school, 1862–'82, at the time Charles O. Whitman was there and had preceded him there probably ten years. Leonard was especially interested in mathematics and during the later years of his life devoted much time to "squaring the circle," *i. e.*, to determining if the relation π can be exactly expressed by a fraction. He is said to have worked out the decimal to over 1,250 places. Relatives say that George Leonard helped secure a college education for Charles and his influence must have been great in getting his parents to let Charles go instead of, as eldest son, helping his father with his business. Leonard graduated, 1859, and taught, probably 1859–'62, at the academy in Norway and that at Paris only a mile or two away; Charles studied at the Norway Academy, 1861–'64. George Leonard, as we have seen, was already a veteran at the English high when Charles came there to teach, retaining his connection with it for seven years, and during this entire period his uncle taught there.

Another instance of scholasticism in this family is that of Charles's first cousin, Rev. Harrison Spofford Whitman, who was born 1844, a year or two later than Charles. Harrison is the son of Harrison Whitman, who was a farmer, at one time captain of an infantry company of Woodstock that saw some service at the time when war was threatened over the boundary of Maine; he was also some time coroner of Oxford County, and died at the age of thirty-one years, leaving a widow and three children. Harrison, Jr., early showed remarkable aptitude in composition (as did his brother and sister); he wrote both prose and poetry; he was fond of study, graduated at Bowdoin in 1869, teaching school meanwhile to earn his tuition and expenses, was for two years principal of the Thomaston Academy, '69–'71; then taught mathematics and later classics at the Dean Academy, Franklin, Massachusetts, '71–'74; then he studied theology at Tufts Col-

lege, graduating 1877. He has ever since taken a leading position in the Universalist ministry of Maine, having been pastor at Augusta and being now at Portland, Maine. He is an able preacher and popular among his parishioners.

This brief account of close relatives who have been distinguished scholars makes it clear how Charles O. Whitman found it natural to follow a scholarly career.

Thoughtfulness and Classicism.—Not all scholars are of the same type. In one type (the hyperkinetic or romantic) there is a rich flow of brilliant ideas and a rapid passage from one subject of interest to another. In the other type (the hypokinetic or classic) there is a profundity of consideration of a subject and a persistence of interest in it. Charles O. Whitman belonged to the latter type. This type ordinarily shows a recessive inheritance. His mother apparently showed this type. She was gentle and pleasant and never lost her even temper; while in many of the Whitmans the temper was of the periodically explosive sort; but there is evidence of the classic temperament on the paternal side, *e. g.*, in Harrison S. Whitman.

That Charles Whitman was of the classic type and was thoughtful will be generally conceded; he was, indeed. one of the best examples of this type that one could find. He felt little pressure to express himself. His principal biographer (Lillie, 1911) records only 67 titles of which 7 are his annual reports,—reports that, toward the end of the series, were secured only with much difficulty and after long delay, and for the last twelve years not at all. Of the remaining 60 there are hardly 20 that are to be classed as typical professional papers, giving the final results of finished observation. Some of the papers are brief notices of technical methods (Methods in the Zoological Station in Naples, 1882, with a French translation; treatment of pelagic fish eggs, 1883; means of differentiating embryonic tissues, 1885; osmic acid and Merkel's fluid, 1886). Other papers are polemical (new facts

about the Hirudinea critique of Apathy, 1888; Apathy's grief and consolation, 1899).

A group of papers (9) of a semi-popular sort relate to the work and aims of the Biological Laboratory. Most of the remaining non-technical papers are delightful essays, chiefly upon philosophical biological matters. Such are: "The Seat of Formative and Regenerative Energy," 1887; "The Naturalist's Occupation," 1891; "The Inadequacy of the Cell Theory of Development," 1893; "General Physiology and Its Relation to Morphology," 1893; "Evolution and Epigenesis," 1895; "Bonnet's Theory of Evolution; a System of Negations"; also "The Palingenesia and the Germ Doctrine of Bonnet," 1895; "Animal Behavior," 1899; "Myths in Animal Psychology," 1899.

The more strictly investigational papers fall into three periods: (1) The invertebrate period—devoted chiefly to the leech, *Clepsine,* which was the subject of his doctor's thesis and upon which he wrote more or less from 1878 to 1899—a period of 21 years. Here also belongs his Naples work on Dicyemids. (2) The period of vertebrate embryology, beginning with work done with Alexander Agassiz on pelagic fish eggs, 1883–1889, on amphibian eggs. 1888, and the ganoid fish, *Amia,* 1896. (3) The period of genetics, foreshadowed in his note "Artificial Production of Variation in Types," 1892, and continued with the pigeons to the end, 1910, in all eighteen years. In his work with worms, amphibians and pigeons he was led to reflections upon animal psychology and to the publication of his classic paper on animal behavior, 1898, and his brief paper on myths in animal psychology, 1899.

Many of these papers are highly finished and give the results of prolonged contemplation. Professor Whitman. especially in his later years, repeatedly spoke disdainfully of rushing into print and making an annual "dump" of scientific gleanings—and these were the natural expressions of his own nature; perhaps he insufficiently recognized that all persons were not constituted like himself and could not react in the same way. Whit-

man, indeed, planned to publish far more than he did; he had accumulated materials that were nearly ready for the printer. But the absence of the hyperkinetic *drive* combined with the manifold duties of the moment led to procrastination for the more convenient period of prolonged quiet which an overactive world never afforded him. On the other hand, had it not been for the exceptional pressure brought to bear on him for an address or a contribution we might have had less than we have from his pen. Of his writings Lillie truly says (p. lxxiii):

> His published papers, mostly short, are models of condensed thought, written in a fine, polished, characteristic style. No less care was devoted to the form than to the substance, and some of his papers will certainly endure as classics of the biology of his time. . . . He rarely had occasion to correct any published statement, and even less rarely, perhaps, to change in any radical way a point of view to which he had once committed himself.

Conservatism, so frequently associated with hypokinesis, was marked in Whitman. He was not very cordial to developmental mechanics, and was critical of the enthusiastic rush to the mutation theory and Mendelism. He could not easily abandon old ideas for new, and ally himself with the latest biological fad.

A strong philosophic and argumentative tendency is found on both sides of the house. His mother's father was much given to theological discussion, and was an ardent adventist. Charles's father, too, was argumentative. The philosophic tendency in Whitman was marked in his writings. In one of his manuscripts of Westford days, at the age of 26, he discusses the topic "Progress Has No Goal" and again "Womanhood Suffrage," of which the tenor is shown by the concluding sentence:

> Female suffrage . . . may meet with opposition, as indeed, every reform does, but all this opposition is but the alarm of the great clock of human progress, which is soon to strike the hour when all enlightened nations shall recognize not only manhood but also womanhood suffrage.

This is quoted as an illustration of youthful style and not a statement of his views in later life.

Whitman's hypokinesis shows itself even in his handwriting. It altogether lacks the running dash of the

hyperkinetic. Each word is worked out with some effort. It is interesting to compare his chirography of Westford Academy days (when he was about twenty-six) and that when he was fifty-six (Fig. 14). There is the same loop from the end of the word to cross the "t" in "the"; the same scanting of the terminal "y"; the same form of the capital "I." In thirty and indeed in the course of forty years his handwriting showed no important change.

Deliberateness.—Professor Whitman's movements and speech were characterized by deliberateness—another characteristic of the classical type. It is, of course, a mere caricature to say, as an unkind critic once did, that his lectures consisted of pauses punctuated by sentences. I mention this because it brings vividly to mind a way he had in lecturing or addressing his seminar students to pause frequently for some seconds looking pleasantly over the room before beginning the next sentence. Even in conversation he would turn a calm, thoughtful face toward you and express himself clearly and deliberately. So marked a deliberateness is not shown by his sibs, but his brother shows something of it, and I am told that his father was slow of speech.

Literary Ability.—While Whitman did not have a strong internal impulse to write, what he published is mostly characterized by high literary finish. Speaking of cooperation between the organic and the inorganic sciences he says (1895, p. iv):

> Comparison of standpoints must benefit both sides. Cross fertilization works rejuvenation in theories as in organisms. The biologist may pause to see how the individual vanishes in the abyss of the universal, and how self determination dissolves in the pressure of the physicist's fundamental postulate of inertia. The physicist may find it agreeable from time to time to turn from the Nirvana where self and not self, rocked in blissful reciprocity of vibration, annul each other, to the world where self asserts itself in organic determinations, issuing in purposeful adaptations and conscious, intelligent action.

Again, speaking of Bonnet he says:

> With a zeal never daunted, and an ingenuity seldom baffled, never defeated, he piled mountain upon mountain of negation, rolling Ossa upon Olympus and Pelia upon Ossa, until the whole organic world

seemed to be completely buried under a stupendous mass of negations, blinding in one infinite negation—*No Change.*

Perhaps something of Whitman's interesting and vivid style may be referred to the influence of his teaching of English in the high school; but much of it appears in papers written before the Boston days. In them, too, he frequently uses the rhetorical question so often found in his later writings (cf. Fig. 14, upper). I think we must conclude that this literary ability has a constitutional basis. We have seen above that his cousins, children of Harrison Whitman, "early showed a remarkable aptitude for composition both in prose and poetry." A granddaughter of Chauncey Whitman, brother of Charles's father, was an authoress of poetry, for which she found a market.

Pertinacity.—In Whitman's combination of traits was found an element of pertinacity that was at times very formidable. Had it been less he could hardly have successfully overcome the handicap of comparative poverty, despite which he went through college. It showed itself again when he insisted in Japan that his student's papers should be published under their own names: and, when, since he was overruled, he resigned his professorship. It showed itself again in his struggle with a minority of the trustees of the Biological Laboratory, in which his views prevailed. It showed itself still again in his relations with Clark University, which led to his acceptance of the offer to go to Chicago. In minor departmental and laboratory affairs, as his colleagues well recall, this gentle-mannered man would show at times uncommon resistance to suggestion and persuasion. One, therefore, learns with interest that his father also was set in his opinions and could not readily be made to change his views. Indeed, Charles is seen to be an interesting mixture of gentleness, as shown also by his mother, and tenacity of purpose, as shown also by his father, his mother's father and other members of the family. Closely akin to his insistence on his ideals was his uncompromising disposition. As Lillie well remarks,

It is questionable whether his life would have been so valuable, had his disposition been more pliable.

One other trait that sometimes showed itself, especially in his writings, was his capacity for trenchant and satirical criticism. Morse (1912, p. 283) has given some examples. It is characteristic of many hypokinetics to feel deeply and to resent warmly. In weighing any criticism we must always consider the personality of the critic. Perhaps in this reaction of Whitman's we see trace of a Whitman "sternness" shown also by his father.

Artistic Taste.—Professor Whitman had a keen artistic sense. This is proved by the success, in his boyhood, of his mounts of birds. I think we may go back of this and find evidence of an artistic sense in the appeal made to him by natural beauty—the beauty of forests, flowers, birds and beasts. To a person without the sense of beauty natural forms have little attraction. Later this sensitiveness to and love of form shows itself in the beautiful drawings he made at Penikese, the exquisite plates of leeches and the delicate pencil drawings of the Dicyemids. It is clear that the art of Japan appealed strongly to him and he had in his house at Chicago many examples of that art. It was love of form that made him a *morphologist* and led him in the *Journal of Morphology* to introduce a beauty of execution of plates exceeding anything then current in America. It was this sense of beauty that led him to select excellent Japanese artists to draw and paint his pigeons. While I have not been able to make an exhaustive study of the distribution of artistic sense in his relatives, it appears that his father, who was a carriage manufacturer, was an artistic one. The woodwork of the finish was done very carefully, the wheels made by hand and so artistically was the whole executed that he once received a silver cup as prize for one of his carriages at a competitive exhibition. On the other side we find his mother's father, Solomon Leonard, was also an artisan. He established an iron foundry at Pinhook and his kitchen ware was so satisfactory that "the name of Solomon Leonard was known in every household." When later he retired from business he maintained "a small furnace at Bryant's Pond, where he made small castings to pass away the time." So it seems probable

that a taste for the beautiful in form and color had a constitutional basis in Charles.

Musical ability is frequent in the Whitman side of the house. Nearly all of the Whitmans could sing; especially good were his father's brothers. But Marcia Leonard, the mother, could not carry a tune; and the children were apparently not good singers. Charles tried to learn to play the melodeon when he was a boy, but had no aptitude for it; his elder sister, however, plays well on the piano and her son is a performer on the violin, of great ability, and a brother of Charles plays the violin well.

Of *mechanical ability* Dr. Whitman had more than the average man. His success in mounting birds (in his father's carriage shop) required manual dexterity. His sister recalls that he made a martin house. This ability shows itself again in his interest in methods of microscopical research which culminated in a volume—the only book he ever wrote—entitled: "Methods of Research in Microscopical Anatomy and Embryology," 255 pp., Boston, 1885. Of this book 55 pages are devoted to instruments and methods of imbedding, including 28 figures of machinery and apparatus, and pages of descriptions of the principles and details of this apparatus. It is improbable that Whitman would have collected such materials and written such a book had he not been interested in and had an insight into mechanical devices. This taste he maintained to the end, and he tarried long over a student who had invented some new instrument.[5] This mechanical insight enabled him to do things in ship-shape fashion. His charts and general arrangements of his laboratory and breeding pens showed this *savoir faire*. With less mechanical skill the feat of moving his pigeons to Woods Hole and back each year would hardly have been feasible. Whitman came from a race of artisans on both sides. His father was a carriage maker; his father's sister Loney had a son, Edwin, who is a machinist and another, Edson, who is overseer in a fabric mill. One of his uncle Cyprian Whitman's sons is an engineer. Of Elhanan Whitman's sons, Austin was a farmer with more

[5] Dr. Oscar Riddle.

than average mechanical ability and Edgar was a carpenter and always ingenious. Even Elhanan's daughter, Mary, says she is handy with tools and so are all of her sons. On the mother's side we have doubtless mechanical interests in at least the grandfather, Solomon Leonard, the iron founder.

The social instinct was highly developed in Whitman. While he rarely appeared at social functions and seemed to shrink from exercising the presidential office to which learned societies repeatedly elected him, yet he liked the society of his younger colleagues, frequently had informal lunches or dinners and, after the excellent meal, would encourage the guests to sit and talk on the matters that were uppermost in the minds of such young and ardent students of biology.

Given a young man of good health, fairly ambitious, dogged, scholarly, gentle, friendly, philosophical, conservative, with an ardent love of natural objects, literary and artistic ability of a high order, and with a *savoir faire* and place him in an environment of a scholarly uncle who is in a position to assist his nephew; of the stimulus of magnetically charged biological atmospheres at Penikese and at Leipzig; of an epoch when new machinery and new methods are being rapidly introduced into a new science; of a period in a young science that demanded organization of laboratories, university departments, societies and journals; and the reaction is that of the propositus; even though handicapped (?) with a hypokinetic temperament, an uncompromising attitude and, in his earlier years, an occasional tendency toward caustic criticism. At any rate such was the man, such the environment that provided the stimulus, and such the life history of Charles Otis Whitman.

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MENDELIAN FACTOR DIFFERENCES VERSUS REACTION SYSTEM CONTRASTS IN HEREDITY[1]

T. H. GOODSPEED AND R. E. CLAUSEN

During recent years there has been a remarkable advance in our knowledge of Mendelian principles of heredity. This advance has for the most part had its source in the important and fundamental work of Morgan and his associates (1915) in which they have been concerned with the mutations of the common fruit fly, *Drosophila ampelophila*. The results of other work, in so far as agreement has permitted, have been brought into harmony with the principles arrived at through these investigations. As a result there has been developed a fairly clear and comprehensive conception of the constitution of the hereditary material and the nature of the mechanism by which it is distributed in gametogenesis, a conception which furnishes a consistent explanation of the products of Mendelian studies.

Morgan has stated that the fundamental principle of Mendelism may be reduced to this, that the units contributed by two parents separate in the germ cells of the offspring without having had any effect on each other. This conception of the absence of any factorial variability save that concerned in the discontinuous changes in factors involved in mutations has furnished the working hypothesis for Morgan's brilliant analysis of the germ plasm of *Drosophila*. Although the results of the *Drosophila* investigations have been ably presented elsewhere (Morgan et al., *l. c.*) it seems well briefly to review them here especially as they are vital to the argument presented in this paper.

[1] The investigations herein reported have been assisted by that portion of the Adam's fund granted to the department of botany by the department of agriculture of the University of California.

The large amount of data which Morgan and his associates have collected within the past six years has clearly demonstrated that the chromosome mechanism furnishes a basis upon which the behavior of Mendelian units may be logically and consistently explained. In an investigation of *Drosophila* over a hundred factor mutations have been discovered and studied, and these have been found to fall into four groups with respect to the linkage relations they display with one another. These four groups correspond to the four pairs of chromosomes. By determining the linkage values which are displayed within groups it has been possible to demonstrate that there is a consistent, invariable, linear arrangement of factors within the chromosomes at some time in their history. From this data Morgan and his associates have been able to prepare a map of the relative positions of the factors in the chromosomes. The complete conception, therefore, pictures the chromosome at some stage in its history as a linear series of loci. When a change occurs in some locus, a corresponding change of some sort may occur in somatogenesis, so that the individual which develops from such a set of factors with the changed locus differs in some particular way from an individual which develops from the normal unchanged series of loci. The change in the characters of the individual will depend not only upon the particular locus which has been changed, but also upon the particular way in which that locus has been changed. A changed locus, however, maintains the same position with reference to the other loci as did the unchanged locus, and this fact is the basis of Mendelian behavior, for knowing the behavior of the chromosomes in reduction, it enables us to gain a clear conception of the nature of Mendelian segregation.

When now we consider the particular factors themselves, the changed loci of the system, we see clearly that important physiological relations exist among the various loci. It is an appreciation of this fact that has led many investigators, among them Conklin (1908), Jennings (1914), Morgan (1915 *b*), Pearl (1915), and Wilson (1914),

to insist that the factors can not be regarded as determiners in themselves; but rather that they are differentiators, that working together with other factors in the system a difference is produced in somatogenesis which has its origin in some difference, some change in a locus in the system. For when in *Drosophila* a change in the locus *W* is produced, or in *Y*, such that the individuals developing from systems with these changed loci are white-eyed in the one case and yellow-bodied in the other, it seems evident that the change is more profound than the color of eyes or of body; that beyond these changes there is an underlying, elusive, physiological change resulting in individuals that are less vigorous and less fertile than those which develop from the normal unchanged system. The fact that a factor may have a primary, simple, easily recognizable effect and secondary far reaching effects, the latter to be attributed to the modified physiological relations resulting from a change in one of the members of a system, is one which has often been observed and which is of fundamental significance in our conception of the interrelations of the genetic factors. There are, however, other instances which may be cited of a somewhat similar nature, locus changes which produce certain characteristic effects under particular environmental conditions, but fail to disturb the normal behavior of the factor system when these conditions are not met.

It would, of course, be possible to recount almost indefinitely the specific effects of particular locus changes, whereas evidence concerning these far reaching effects of single factors is too scanty to warrant further discussion of this point. However, Morgan (1915 *a*) has been able to establish the relations displayed by the factor for abnormal abdomen and to demonstrate that only under very particular conditions is the presence of this genetic factor manifested by its characteristic expression, and that when these conditions are not present the product of somatogenesis may not differ in appearance from the normal fly, although differing from it both in genetic constitution and

hereditary behavior. We have thus a factor here which evidently has such a relation to the other members of the factor system that only under peculiar environmental conditions does it disturb the normal course of somatogenesis. Miles (1915) has likewise investigated a type of chlorophyll reduction in maize in which the recessive forms are yellow seedlings which usually show a distinct greenish tinge at the tips of the leaves. This type of chlorophyll reduction displays normal Mendelian behavior in inheritance giving in the progeny of heterozygous plants a ratio of approximately three seedlings which are of the normal green coloration to one which is of the yellowish type. The heterozygous plants possess the normal depth of coloration and can not be distinguished from those which are homozygous for the production of normal chlorophyll coloration. The yellow seedlings on the other hand form a distinct and easily recognizable class with no tendency toward intergradation, an observation which we have ourselves been able to confirm in independent mutations involving this locus. Usually these seedlings die as soon as the food material in the endosperm is exhausted, for under ordinary conditions the change in the locus is incompatible with a normal development of the individual, it is too profound an alteration to give a normally functioning factorial system. Miles found, however, that when the yellow seedlings were grown under particularly favorable conditions, they developed a normal chlorophyll coloration and produced plants which were able to go on through the cycle of changes included in the normal development of the maize plant. Now this behavior can not be referred to any change of the reduction locus back to the original condition, for the progeny of such plants consisted entirely of yellow seedlings; indeed, such a reversion would be inconceivable. Obviously the explanation of the situation will be found only through a consideration of the system with the recessive reduction locus. Normally this unchanged locus performs a definite function in determining the production of chlorophyll in the plant, but this function is

performed in conjunction with a number, perhaps a very great number or even all, of the other loci within the system. With a change in this particular locus, however, comes a change in the normal course of events in chlorophyll production, in that the rate at which the system is able to produce chlorophyll has been altered. Nevertheless, this change does not completely prevent the system under favorable conditions from going on and ultimately developing the same reaction end product which would have developed in the normal unchanged condition, but more rapidly.

Among such factors as have a profound influence upon the interrelations within the systems of which they are a part are those which Morgan (1914) has called lethals. Morgan's work with lethals is particularly suggestive because he has been able to demonstrate that they, like other normal Mendelian factors, occupy a definite locus in the chromatin system and display the same perfectly definite and consistent behavior with reference to the other loci of the system as do all other changed loci which do not interfere with the normal development of the individual. It is entirely possible that some of the lethals, like the chlorophyll reduction locus which we have discussed above, may yield systems which occasionally permit of the normal development of the individual, at least certain peculiar sex ratios which have been obtained might indicate that fact (Morgan, *l. c.*); but the important result of these investigations with lethal factors lies in that fact that certain kinds of changes in some loci are incompatible with normal functioning of the chromatin system. It might in addition be noted that there seems to be no particular reason why we should not include in the same category with lethals, the type of chlorophyll reduction mentioned above and those other types in maize which result from such profound factor changes that no development is possible after the food supply of the endosperm is exhausted.

Now giving the above results their broader and more general interpretation, it would appear that the factors

make up a reaction system the elements of which bear a more or less specific relationship to one another. It is this specific interrelation of the factors of the reaction system which determines that wheat produces wheat, and corn, corn, and so on through the whole realm of living matter. With this in mind it is at once apparent that normal Mendelian behavior can not be considered as a contrast of different reaction systems, but that in such cases the two organisms contrasted must possess fundamentally the same reaction systems, only a relatively few elements within the reaction system differing, and these not in a fundamental fashion. In fact it seems entirely logical in the light of modern Mendelian developments to consider each particular locus as made up of a definite nucleus, some complex organic compound perhaps, with a number of end chains which may be altered in various ways without changing the structure of the nucleus of the locus. According to this conception the fundamental relation of the locus to the other elements of the reaction system would remain unchanged, while the end product would be altered in some particular manner. There is probably no more striking confirmation of this conception than the suggestive hypothesis of multiple allelomorphs which Morgan and his associates (1915, *l. c.*) have developed. Their results and those of others in this connection seem to show clearly that the explanation of Mendelian differences on the basis of such a profound change as the dropping out of an element from a delicately balanced reaction system is practically out of the question. In multiple allelomorphs we have not one, but several, changes within the same locus. The similar effect which these changes have on certain organs of the body, for example, that relation shown in the locus *W* in *Drosophila* as a consequence of which the normal red eye color may be changed to white, eosin, or cherry depending upon a particular change in the locus, are such as to indicate that these are probably changes around the fringe of the molecule and not such as fundamentally affect the structure of the entire locus. Moreover, the relations thus exhibited again indicate that

the locus has a particular place and function in the reaction system, that it bears a specific relation to the other elements of the system.

It is true that investigation in plant breeding has not yet progressed far enough to furnish a definite confirmation all along the line of the work with *Drosophila*. The reasons for this are very obvious and they lie in the technical difficulties involved in such work rather than in fundamental disagreements in principle. It has not as yet been possible to study as many factors in any plant species as have been investigated in *Drosophila*, nor to carry the work through as many generations nor to employ as large populations. Moreover, most plant material is more difficult to handle from the standpoint of a chromosome analysis on account of the longer period of time necessary to secure data and the greater amount of attention which must be given the cultures and the larger number of chromosomes which are usually involved in such species. It must also be borne in mind that practically all the *Drosophila* differences have arisen under observation as simple factor mutations, and it has therefore been relatively easy to determine their relations to the other factors in the system. In plants, on the other hand, the material has presented itself as a confusing array of varieties containing for the most part a large number of recessive factors, and usually the original form from which they were derived, corresponding to the *normal* fly of Morgan's work, has not been obtainable and would not have been particularly useful, had it been available. Nevertheless, there appears to be no real difficulty in the way of accepting the conception derived from the *Drosophila* studies as a definite, consistent working hypothesis; for it is difficult to believe that the behavior of plant material should be fundamentally different, and indeed points of correspondence are not lacking to warrant us in viewing somewhat sceptically any undue emphasis placed upon the differences which may seem to obtain.

We may now return to the conception of the reaction system as a unit in itself in the sense that it is made up of

a large number of elements which bear a more or less specific relation to one another. This is the important physiological conception which has grown out of the vast amount of work which has been done in recent years in the analysis of the hereditary material. This is no new contention; it has been advanced and ably advocated by many investigators, but we feel that certain consequences of this conception have not been given the consideration their importance deserves. For if this conception be valid then it should not be possible, in certain cases at least, to shift and recombine the elements from which systems have been built up in the haphazard way that some advocates of Mendelism have attempted to do. If, for example, it is possible to obtain hybrids involving not a contrast between factors within a single system, but a contrast of systems all along the line, then it is obvious that we must consider the phenomenon on a higher plane, we must lift our point of consideration as it were from the units of the system to the systems as units in themselves.

Our attention has been called to this extension of the Mendelian conception by the behavior of species hybrids of *Nicotiana* which have been studied at the University of California during the past six years. This study has been concerned particularly with hybrids between *N. sylvestris* and varieties of *N. Tabacum*. These species, the former represented in the collections of the University of California Botanical Garden by a single type and the latter by a considerable variety of distinct forms, belong to entirely distinct sections of the genus *Nicotiana* and differ in important particulars which have been described elsewhere (Setchell, 1912). Goodspeed (1913) has studied a large number of different reciprocal hybrids between *sylvestris* and various of the distinct varieties of *Tabacum*. These hybrids are all partially sterile. It is possible to obtain a few viable seeds from open pollinated flowers and from those pollinated with *Tabacum* and *sylvestris*, but it has never been found possible to obtain any selfed seed. The phenomena displayed by these hybrids in development and inheritance admit of a consistent ex-

planation, if we regard them as the outcome of a contrast of two distinct Mendelian reaction systems the elements of which can not be freely interchanged without profoundly affecting the general functions of the reaction systems thereby resulting. We shall take up in very general fashion the points which have inclined us to this view, reserving for a later treatment the discussion of the hypothesis in detail and also the presentation of the extended data.

When hybrids are obtained between *sylvestris* and the various varieties of *Tabacum* they agree throughout in F_1 in presenting the entire set of characters of the *Tabacum* parent to the exclusion of those of *sylvestris*. This behavior may be definitely accounted for as a dominance of the *Tabacum* reaction system as such over the *sylvestris* reaction system. For point by point and character by character throughout, the correspondence between the *Tabacum* variety and its F_1 *sylvestris* hybrid may be demonstrated in a remarkable fashion and this irrespective of whether the factors concerned in these character expressions in the *Tabacum* varieties are dominant or recessive in varietal crosses. This correspondence is not only apparent in general appearance, but it extends to minute details of form and structure, and it is displayed even in the more intangible characteristics generally included under the term habit—*i. e.*, such characteristic varietal peculiarities of expression as the method of branching, insertion and inclination of the leaves, the type of inflorescence, and so on through a whole series of details. For example, when *N. Tabacum* var *macrophylla* (Setchell, *l. c.*, p. 8) is the *Tabacum* parent, the F_1 hybrids display the particular appearance and also the particular characteristics of *macrophylla,* except that throughout they are expressed on a very much enlarged scale (*cf.* East and Hayes, 1912). The broad clasping leaf of *macrophylla* with its distinctly pointed tip is faithfully reproduced in the hybrids. The flowers show no effect of the very much elongated corolla tube and the lobing of the limb peculiar to *sylvestris,* but display the *macrophylla*

proportions throughout. The stout tube, swollen infundibulum, and pentagonal limb are clearly derived from *macrophylla,* and the color is rose red of approximately the same depth and tone as that of *macrophylla* and in striking contrast to the pure white of *sylvestris*. In habit the hybrids resemble *macrophylla*. In early growth they are not characterized by the long maintained rosette which is so characteristic of *sylvestris,* and in leaf distribution, branching, and type of inflorescence they again correspond to their *macrophylla* parent.

When an entirely different set of characters is concerned in the *Tabacum* parent the F_1 hybrid with *sylvestris* is still an exact replica of the particular *Tabacum* used. For instance *N. angustifolia* (Setchell, *l. c.*, p. 9) and *sylvestris* give a hybrid which is entirely different in general appearance and all details from that obtained between *macrophylla* and *sylvestris,* and which displays throughout the *angustifolia* characters. The leaves of the hybrid are obliquely ovate-lanceolate and taper gradually to a long, curved point. They are also distinctly petioled like those of *angustifolia*. These characters are in striking contrast to those characteristic of the leaves of *sylvestris,* which are broad throughout, broadly pointed, and have a broad clasping base. When flower characters are examined, *angustifolia* is again faithfully reproduced in the F_1 hybrid for its flowers have the slender, straight corolla tube with practically no suggestion of an infundibulum and the deeply divided limb with narrow lobes that taper into long slender tips, all so characteristic of *angustifolia*. Like those of *angustifolia* the flowers are pink. In habit these hybrids again resemble *angustifolia*. This resemblance is displayed in a particularly striking fashion in the graceful, drooping manner in which the leaves are borne in marked contrast to the stiff, erect manner in which the leaves are borne by *sylvestris*. Throughout, *macrophylla* and *angustifolia* present a strikingly contrasted set of characters, yet in each case they are reproduced in their entirety in F_1 of the hybrid with *sylvestris*.

When particular tagged Mendelian factors are considered the same behavior is displayed. Perhaps there is no more striking instance of this than that shown by the expression of the calycine flower type in these *sylvestris* hybrids. When *N. Tabacum* var. *calycina* (Setchell, *l. c.*, p. 6) with its peculiar split, hose-in-hose flowers is crossed with *Tabacum* varieties of the normal flower type, the F_1 hybrids display the normal flower form and segregation occurs in F_2 into normal and calycine in accordance with simple Mendelian expectations. But when the *Tabacum* reaction system carries the recessive calycine flower factor into these species hybrids with *sylvestris* then every flower on the F_1 plants displays a more or less calycine structure. Similarly when the parthenocarpic characteristics of *N. Tabacum* var. "Cuba" (Goodspeed, 1915) are carried in by the *Tabacum* parent then the F_1 hybrid, instead of shedding its capsules soon after anthesis as is the case in all the other *Tabacum-sylvestris* hybrids, retains them indefinitely, in spite of the fact that no good pollen is produced, and thus non-fertilization, the stimulus for fruit abscission in *Nicotiana,* here also is the rule. So far as present evidence indicates this characteristic is rather strictly confined in *Nicotiana* to the variety "Cuba." This behavior of recessive factors of *Tabacum* varieties in hybrids with *sylvestris* is a striking confirmation of the conception that in such cases there is a contrast between distinct reaction systems rather than between certain factors as opposed to each other. In general when *Tabacum* varieties of the type mentioned above are crossed with each other the hybrids, especially with respect to flower color, leaf shape, etc., are intermediate. The contrast in this case is not one between two distinct Mendelian reaction systems, but it is merely a contrast of certain differences within a common system, and the segregation in subsequent generations, although complex, indicates a general accordance with normal Mendelian expectation. But in the case of species hybrids between *Tabacum* and *sylvestris* the contrast is between distinct

Mendelian reaction systems and the consistent reproduction of all *Tabacum* characters, whether qualitative or quantitative, indicates at one and the same time that these are fundamentally of the same nature, depending essentially for their expression on a complex set of Mendelian factors, and, moreover, that the *Tabacum* system as a unit dominates the course of somatogenesis and determines the reaction end products of the two systems.

This domination of the somatogenic processes by the *Tabacum* reaction system is followed by important experimental possibilities. If the species hybrids always display the *Tabacum* characteristics as completely as all our present evidence indicates that they do, then they will furnish a powerful method of attack on the problem of Mendelian behavior in the *Tabacum* section of the genus *Nicotiana*. For by crossing hybrids between *Tabacum* varieties with *sylvestris*, it should be possible to secure in the partially sterile hybrids resulting a phenotypic reproduction of the gametic series of the *Tabacum* parent. This series would not be complicated by intergrading of heterozygous forms, because the plants thus obtained would exhibit the phenotypic characters of homozygotes, and recessive factors as well as dominant ones would be reproduced in their proper place in the *Tabacum* system. Apparently it should be possible, therefore, to demonstrate the fundamentally similar nature of linkage in *Nicotiana* and *Drosophila*. Such an analysis will still be very difficult in *Nicotiana* on account of its high chromosome number, but by the method of procedure outlined above some distinct advance at least seems perfectly feasible. These, however, are matters on which we have as yet very little data.

Since, therefore, the *Tabacum* reaction system dominates the somatogenic processes in the hybrid to nearly or quite the exclusion of the *sylvestris* system, the elements of the two systems must be largely mutually incompatible. Free interchanges between the two systems would not, therefore, necessarily result in the formation

of functional Mendelian reaction systems. This high degree of mutual incompatibility of the two reaction systems exhibits itself in the high degree of sterility of the F_1 hybrids. As Goodspeed (1912, *l. c.*) has shown, however, this sterility is only partial and a few good ovules are formed which produce viable seed in the case of open pollination or when crossed back with the parents. In evidence which is presented elsewhere (Goodspeed and Ayres, 1916) it has been shown that, while it is experimentally possible to modify the behavior of the F_1 plants in such a manner that the fruits without pollination are retained for a considerable period rather than falling soon after anthesis, the percentage of good ovules produced can not be appreciably modified. This is an important point, for it indicates that the number of good ovules produced is a function of the chromatin behavior and not to be influenced by environmental factors, and that they should, therefore, exhibit a consistent behavior and lend themselves to a logical interpretation. In fact, evidence at hand indicates that the small percentage of functional ovules represents the *Tabacum* and *sylvestris* extremes of a recombination series, and that, therefore, the middle members of the series, which are made up of relatively high proportions of both *Tabacum* and *sylvestris* elements, fail to function because they produce incompatible reaction systems. This is shown clearly by back crosses which have been made with the parents, although here portions of our evidence are not so well controlled as we would prefer. When back crosses are made with *sylvestris* as the pollen parent there is produced a variety of forms many of which are highly abnormal, but among them there is a considerable proportion of plants which are pure *sylvestris* in all characters. These plants are fertile and have bred true to the *sylvestris* type for three generations. A number of the remaining plants resemble *sylvestris*, but show contamination with other elements presumably derived from the *Taba-*

cum reaction system. These contaminations affect the whole plant, not alone any particular character complex. All plants except those which were of the pure *sylvestris* type were sterile. Until this year we have not been able to secure seed from back crosses with the *Tabacum* parent, but from open pollinated seed a variety of forms is produced, practically all of which are of the *Tabacum* type in general appearance. This result is evidently due to pollination of the F_1 flowers with pollen from the wide series of *Tabacum* forms which, in predominating numbers, have always been grown in the cultures. Some of these plants resulting from uncontrolled pollination were likewise fertile. They have been grown for several generations and although displaying segregation, this segregation has never involved the production of *sylvestris* characters, but has been of a type normally found within varietal hybrids of *Tabacum*. The sterile forms in this series largely resembled the F_1 hybrids of *Tabacum* and *sylvestris*, and the occurrence of a few aberrant and *sylvestris* forms which were obtained from the sowing of the open pollinated seed are what would be expected, if pollination was sometimes effected with *sylvestris* pollen.

It appears, therefore, that for these species hybrids the conception of the factors as making up for each species a reaction system in which the elements have a specific relation to one another harmonizes the results obtained with the more recent Mendelian developments. The objection which might be made that interchanges of factors which behave normally in one system should not logically be followed by such profound disturbances as to completely prevent the formation of a functional reaction system is met by several counter considerations. In the discussion of lethal factors it has been pointed out that Morgan (1914, *l. c.*) has demonstrated that changes in many loci of the *Drosophila* system have been followed by failure of the resulting individual to develop. It is entirely conceivable that, if a certain factor *A* in one system

be considered, the corresponding factor A' in the other system, if there be such a factor, might be just as different from A as a lethal factor is different from its normal allelomorph. Further, from a modern Mendelian viewpoint there is no basis for assuming that recombinations could be obtained involving only exchanges in isolated loci in the systems. For if the behavior in segregation in the F_1 hybrids corresponds to that in *Drosophila* then such combinations as could be obtained would depend on the shifting of entire choromosomes or in case of crossing-over of relatively large portions of chromosomes. The recombinations obtained in the hybrids between *Tabacum* varieties and *sylvestris,* provided chromosome distribution takes place after the normal fashion in these hybrids, would involve, therefore, for the most part, the formation of systems containing either whole chromosomes or large sections of chromosomes of opposing systems. Such attempted reconstruction of systems might well fail in cases of any marked specificity in the relations of the factors of the opposing reaction systems, since any large proportions of both systems in a gamete might conceivably destroy the continuity or the balanced relations necessary for the continuance of system reactions. When the proportions of one or the other system are relatively small, the system reactions might be merely disturbed, resulting in the production of the abnormal forms which have been secured in our cultures. It is to such relations between the two systems involved that we ascribe the selective elimination of the greater portion of the possible gametic combinations in the crosses between *Tabacum* varieties and *sylvestris,* and which, therefore, results in a high degree of sterility in these hybrids.

It is of course obvious that there are many categories of sterility, in the hereditary sense as well as in the physiological sense. The particular type herein considered is that which results from relatively wide crossing such as is involved in species hybrids. That certain types of steril-

ity are due to specific factors which display consistent Mendelian behavior has been demonstrated by Bateson and Punnett (1908) and others and has been suggested by Correns (1913) for cases which are concerned with the specific category of normal self-sterility in *Cardamine pratensis*. It appears, nevertheless, as East (1915) in principle has suggested, that many cases in which sterility has followed rather wide crossing would seem to be susceptible of a more logical treatment from the standpoint of non-specific disturbances in the reaction systems involved.

(*To be continued*)

COMPARATIVE RESISTANCE OF PRUNUS TO CROWN GALL[1]

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IN making a study of the plant disease popularly known as crown gall, plant tumor, or plant cancer, it seemed desirable to ascertain the relative resistance of the different species of *Prunus* to this disease. It was soon evident that the usual methods employed in discovering disease resistance would be of little value. The cause of the disease, life history and pathogenic nature of the organism had already been studied by Dr. Erwin F. Smith[2] and his assistants of the United States Department of Agriculture. They showed, by artificial inoculations, the wide range of plants susceptible to infection and also found that some were apparently resistant. Their experiments encouraged the writer to follow with slight modifications the method of artificial inoculations on a number of species and varieties of the genus *Prunus*. It was hoped that suitable resistant stock might be discovered that would be adapted to the propagation of the stone fruits.

Before considering in detail the methods employed, the general characteristics of the disease will be briefly given. The affected part of the tree, shrub or plant is generally found a little distance beneath the surface of the soil at the crown or point where the roots are given off from the trunk. The disease is characterized by an enlargement or gall more or less spherical in shape and consisting of tissue that is usually much softer in texture than normal. The surface may or may not be covered with a normal bark. This enlargement is now known to be caused by a stimulus that comes from the presence of a definite motile bacterial organism known as *Bacterium* (*Pseudomonas*) *tumefaciens*, which lives within certain of the plant cells in relatively small numbers.

Considerable attention was given to perfecting methods for

[1] Paper No. 28, Citrus Experiment Station, College of Agriculture, University of California, Riverside, California.

[2] United States Department of Agriculture, Bureau of Plant Industry, Bulletins No. 213 and 255, 1911, 1912.

the determination of the relative resistance of the different species of *Prunus*. Most of the different species and varieties were budded or grafted on other stock, the scions or bud wood being secured from several reliable sources, such as the Arnold Arboretum, several of the larger nurseries. The methods used are somewhat different from those usually employed in seeking for disease resistance among plants. The plan was to artificially inoculate with pure virulent laboratory cultures the different kind of *Prunus* under experimentation. A number of suscep-

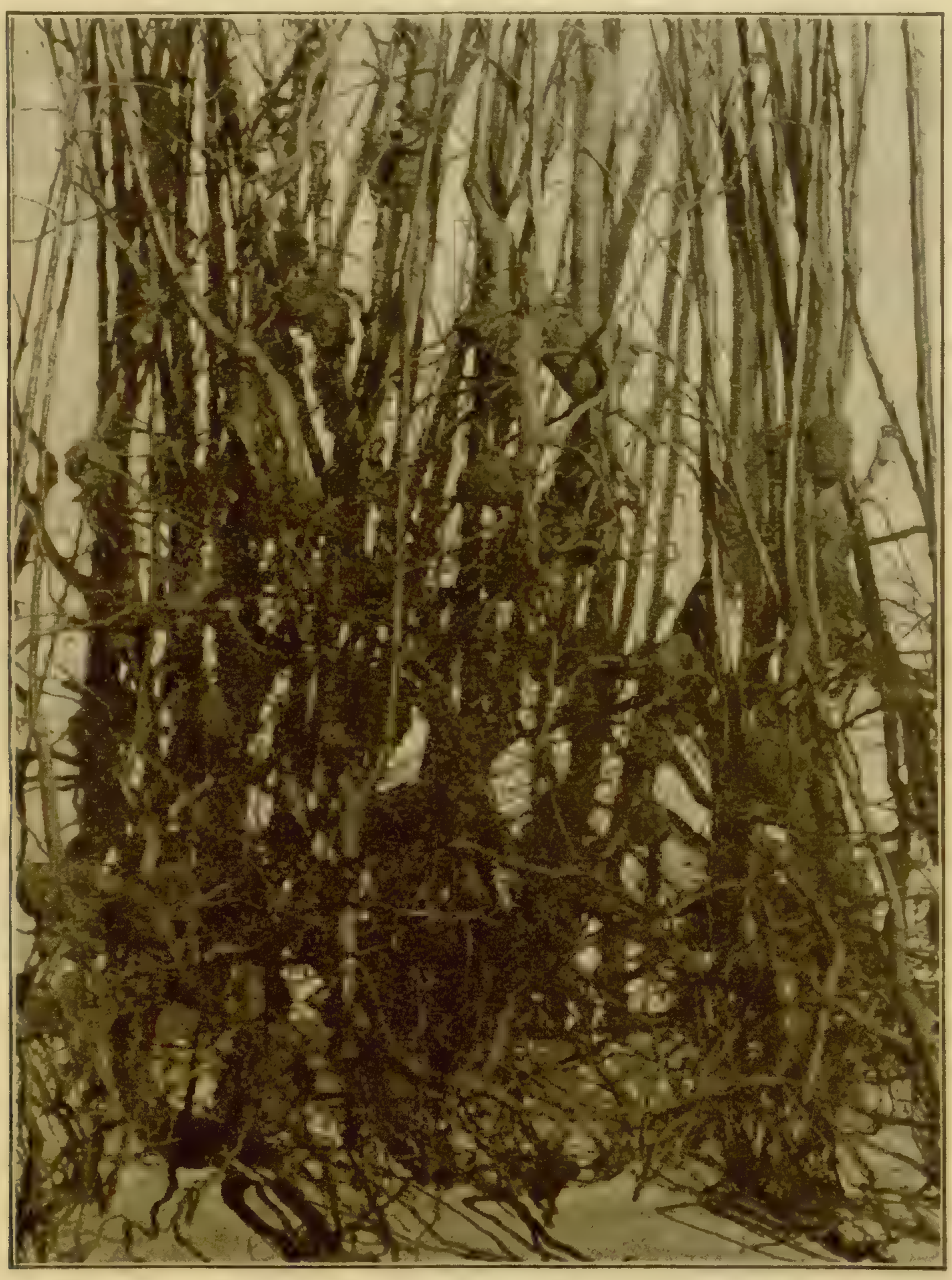

FIG. 1. A bunch of galled peach stock as they often occur in the nursery. Many of the other trees probably had incipient infections unrecognizable at the time of digging, which later developed galls.

tible hosts were always included in the experiment, to act as a check upon the virulence of the culture and any unfavorable climatic condition. In each series of inoculation 5 or 10 punctures were made upon vigorous growing twigs of the current

FIG. 2. Natural gall on English walnut in nursery. This gall appeared at the point where the English walnut stock was grafted. It is common practice to bank the dirt about the scions to prevent drying. The California black root is much more resistant.

year's growth. During the experiments of 1914, ten punctures were always made. This number, being the same in all the experiments, was of material aid in the final compilation of results. Other check punctures were made in the same way as in the inoculations except that none of the organisms were placed in the tissue. An ordinary steel needle in a cedar handle was used in making the puncture inoculations. This was first flamed, then used to convey some of the bacterial growth from the test tube to the twig to be inoculated, the puncture being made through the bark and wood of twig. The organism was grown in a medium made as follows: ½ per cent. glucose, ½ per cent. sodium chloride, ½ per cent. meat extract, 1 per cent. peptone, 1 per cent. agar.

FIG. 3. Two almond roots naturally infected with numerous galls. Such stock would only make inferior trees.

The tubes were incubated from twenty-four to thirty-six hours before being used, at which time there was a vigorous, pearly white, raised growth where the medium was inoculated. The series of inoculations were made a week apart from May 1 to about September 1, 1914. The work of 1913, while similar in nature, was not so extensive as that of 1914. The experiments thus extended over the period of the year when the trees are making their most rapid growth, and should be in their most susceptible condition for infection. The trees were well cared for and made rapid growth during the period the experiments were in progress, and hence were in favorable condition for the development of the disease. No effort was made to protect in any way the punctures, as the use of wax or other covering stimulates callus formation which could easily be confused with the beginning stages of a young gall or with one that has not matured rapidly, as is often the case on inoculated trees showing resistance.

The genus *Prunus* gives a wide range for investigation because of the large number of species and varieties. The following are the species thus far tested by artificial inoculations:

Prunus Allegheniensis, P. Americana, P. Amygdalus, P. andersonii, P. Armeniaca, P. Armeniaca, var. Mikado, *P. Avium, P. Besseyi, P. Caroliniana, P. cerasifera, P. cerasifera,* var. *divaricata, P. cerasifera,* var. *Planteriensis, P. domestica,* several different varieties, *P. eriogyna, P. glandulosa, P. hortulana, P. ilicifolia, P. integrifolia, P. Japonica, P. maritima, P. Mahaleb, P. Mitis, P. monticola, P. Mume, P. munsoniana, P. nigra, P. orthosepala, P. Pennsylvanica, P. Persica,* several varieties, *P. platycarpa, P. pumila* (Linn.), *P. serotina, P. Simonii, P. Watsoni.*

All the above hosts gave positive results from artificial inoculation, except *P. pumila, P. ilicifolia* and *P. Caroliniana.* These three hosts were inoculated during 1913 and 1914 and have always showed negative results. *P. ilicifolia* and *P. Caroliniana* were on their own roots and were not making very rapid growth at the time of the experiment, but it seems almost impossible that they should not have been in a susceptible condition some time during the period from May to September. *P. pumila* made rapid growth, as it was grafted on peach stock, but never showed the least indication of gall formation, nor has it during the ex-

FIG. 4. FIG. 5.

FIG. 4. Artificial gall produced on pepper tree, *Schinus Molle.*

FIG. 5. Artificial gall made by inoculating sour orange seedlings. Citrus stock is only rarely infected naturally with gall, but galls have been artificially produced on lime, lemon, orange and shaddock.

periments of 1915. Only a part of the kinds of *Prunus* just mentioned were thoroughly tested out and these only are included in tabulated results.

It will be noted that Tables II and III represent two varieties of *Prunus domestica*. Not all varieties, however, are equally resistant, but, in general, members of this group are much more

FIG. 6. Artificial galls on *Prunus*. (*a*) German prune, *P. domestica*, a resistant variety. (*b*) Myrobolan, *P. cerasifera*, a susceptible species. Note the difference in size of galls and how in the susceptible kind the galls eventually surround the twig.

TABLE I

A TYPICAL EXPERIMENT WITH *Bacterium tumefaciens*, CULTURE NO. 753 C (JULY 13 TO NOVEMBER 15, 1914), TO ILLUSTRATE GENERAL METHODS USED IN SEARCHING FOR A RESISTANT STOCK. A SIMILAR EXPERIMENT TO THIS WAS MADE EACH WEEK FROM MAY TO SEPTEMBER, 1914.

Experiment 20

Experiment Serial No.	No. of Inoculations	Positive Inoculations	Host	Size of Galls in Inches
745	10	2	German prune	$\frac{1}{8}$–$\frac{1}{4}$
746	10	0	German prune (old)	
747	10	5	Damson	$\frac{1}{16}$–$\frac{1}{8}$
748	10	10	*P. triflora*	$\frac{1}{16}$–$\frac{1}{2}$
749	10	10	Wickson	$\frac{3}{8}$–$\frac{3}{4}$
750	10	9	Burbank	$\frac{1}{2}$–$\frac{3}{4}$
751	10	10	Myrobolan	$\frac{1}{2}$–$\frac{3}{4}$
752	10	10	*P. Munsoniana*	$\frac{1}{4}$–$\frac{3}{8}$
753	10	10	*P. davidiana*	$\frac{1}{4}$–$\frac{1}{2}$
754	10	5	*P. maritima*	$\frac{1}{16}$–$\frac{1}{4}$
755	10	4	*P. dasycarpa*	$\frac{1}{16}$–$\frac{1}{8}$
756	10	0	Golden drop (silver prune)	
757	10	8	Reine Claude (green gage)	$\frac{1}{16}$–$\frac{3}{8}$
758	10	10	*P. Simonii*	$\frac{1}{4}$–$\frac{1}{2}$
759	10	10	Royal apricot	$\frac{1}{4}$–$\frac{1}{2}$
760	10	10	Elberta peach	1–1$\frac{1}{4}$
761	10	0	Bitter almond	
762	10	9	*P. hortulana*	$\frac{1}{4}$–$\frac{3}{8}$
763	10	5	*P. Americana*	$\frac{1}{16}$–$\frac{1}{8}$
764	10	10	*Schinus Molle*	1–1$\frac{1}{2}$
765	10	Positive	Oleander	
766	10	0	*P. pumila*	
767	10	0	*P. Watsoni*	
768	10	8	*P. nigra*	$\frac{1}{4}$–$\frac{1}{2}$
769	10	3	*P. serotina*	$\frac{1}{16}$–$\frac{1}{8}$
770	10	10	*P. institia pendula*	$\frac{1}{2}$–$\frac{3}{4}$
771	10	9	*P. mitis*	$\frac{1}{16}$ small
772	10	10	*P. Mume*	$\frac{1}{2}$–$\frac{3}{4}$
773	10	7	*P. Andersonii*	$\frac{1}{4}$
774	10	6	Duane (Tribble)	$\frac{1}{8}$–$\frac{3}{8}$
775	10	2	*P. Planteriensis*	$\frac{1}{16}$–$\frac{1}{8}$
776	10	8	Myrobolan (Arnold)	$\frac{1}{16}$–$\frac{1}{4}$
77	10	9	Myrobolan (sprouts)	$\frac{3}{4}$–1
778	10	10	Myrobolan (young sprouts)	$\frac{1}{2}$–$\frac{3}{4}$
779	10	10	El Paso	$\frac{1}{2}$–$\frac{3}{4}$
780	10	0	Golden beauty	
781	10	10	Arkansas	$\frac{1}{8}$–$\frac{1}{4}$
782	10	1	*P. Virginiana*	$\frac{3}{16}$
783	10	7	*P. cerasifera divarica*	$\frac{1}{2}$–$\frac{3}{4}$
784	10	2	*P. Besseyi*	$\frac{1}{16}$
785	10	0	*P. ilicifolia*	
786	10	0	*P. Caroliniana*	
787	10	9	*P. orthosepala*	$\frac{1}{4}$–$\frac{1}{8}$
788	10	0	Olive	
789	10	2	*P. Armeniaca* Mikado	$\frac{1}{8}$–$\frac{1}{2}$
790	10	5	Italian prune	$\frac{1}{16}$–$\frac{1}{8}$

so than most other species of the genus. By comparison with other tables, it will be found that the galls are of a much smaller size than on most other hosts. The number of positive inocula-

TABLE II

SUMMARY OF ARTIFICIAL INOCULATIONS ON GERMAN PRUNE, *Prunus domestica*. CONCLUDED NOVEMBER 15, 1914

Experiment Serial No.	Date	No. of Inoculations	Positive Inoculations	Size of Galls in Inches
x 500[3]	5/ 3/14	10	0	
532	5/25/14	10	0	
559	6/18/14	10	0	
x 560	6/18/14	10	0	
617	6/29/14	10	6	$\frac{1}{8}$–$\frac{1}{4}$
x 618	6/29/14	20	0	
667	6/29/14	Check	0	
679	7/ 6/14	10	6	$\frac{1}{8}$–$\frac{1}{4}$
x 680	7/ 6/14	10	0	
745	7/13/14	10	2	$\frac{1}{8}$–$\frac{1}{4}$
x 746	7/13/14	10	0	
x 792	7/20/14	10	0	
870	7/27/14	10	0	
x 871	7/27/14	10	0	
934	8/ 3/14	Check	0	
x 935	8/ 3/14	Check	0	
995	8/ 4/14	10	2	$\frac{1}{8}$–$\frac{3}{16}$
x 996	8/ 4/14	10	0	
A 15	8/10/14	10	0	
A 87	8/15/14	10	0	
xA 88	8/15/14	10	0	
*A*125	8/17/14	10	0	
*A*207	8/24/14	10	8	$\frac{3}{16}$–$\frac{1}{16}$
*xA*208	8/24/14	10	0	
*A*242	8/31/14	10	0	
*xA*243	8/31/14	10	0	
		240	24	

tions as given in Tables II and III, is probably somewhat greater than it should be, as in making the estimate of the number of galls on these resistant stocks, any small enlargement was counted, and subsequent examination has shown that many of these small enlargements have not further increased in size. When a gall becomes established in a resistant variety, it makes rapid growth and eventually forms one of good size. These large galls differ from similar galls on peach and many other hosts in that the gall is attached to a relatively small circumference of the infected twig. The gall growth is often nearly at

[3] Numbers that are preceded by an *x* were made on rapid growing twigs of the current year of a seven-year-old tree. The other inoculations in a young tree two years old from the nursery.

TABLE III

SUMMARY OF ARTIFICIAL INOCULATIONS ON ITALIAN PRUNE (FELLENBERG) *Prunus domestica.* CONCLUDED NOVEMBER, 1913

Experiment Serial No.	Date	No. of Inoculations	Positive Inoculations	Size of Galls in Inches
211	5/10/13	5	0	
216	6/ 1/13	5	2	$\frac{4}{16}$—$\frac{1}{16}$
252	6/13/13	5	0	
264	6/16/13	5	0	
270	6/17/13	5	0	
282	7/14/13	15	5	$\frac{2}{16}$—$\frac{2}{16}$—$\frac{3}{16}$—$\frac{4}{16}$—$\frac{5}{16}$
292	7/13/13	5	0	
315	7/19/13	6	2	$\frac{2}{16}$—$\frac{4}{16}$
328	7/21/13	6	1	$\frac{2}{16}$
332	7/21/13	5	0	
343	7/22/13	5	0	
355	7/25/13	10	4	$\frac{1}{16}$—$\frac{3}{16}$—$\frac{2}{16}$—$\frac{1}{16}$
366	7/30/13	10	0	
259	6/ 9/13	5	0	
375	7/30/13	10	0	
390	8/ 9/13	10	0	
406	8/14/13	10	3	$\frac{2}{16}$—$\frac{1}{16}$—$\frac{1}{16}$
420	8/14/13	5	0	
		127	17	

right angles to the twig which makes these galls stand out for considerable distance from the branch.

It is of interest to note that both of these are prunes that have been under cultivation for many years. The German prune is described as being one of the plums longest under cultivation and the oldest of the prune type. Seedlings also come reasonably true to type which might be of importance if grown from seed for a stock. The Italian prune (Fellenberg) is the popular prune of Oregon and has a history of over a century's cultivation. Further experiments among varieties of the *domestica* group are being carried on. The damson which is sometimes included among the domesticas, shows considerable resistance to artificial inoculation.

Prunus cerasifera, var. *Planteriensis,* Table VIII, is described as a double flowering shrub and is the most gall resistant of any of the tested varieties of *cerasifera,* although this resistance should be again determined. Inoculations in the Arnold Arboretum trees, Table IV, did not develop as many galls as those of the larger Myrobolan tree, either because the former were not

[4] A local commercial stock, propagated in California from sprouts, not the true Duane variety, but a small blue plum having the flavor of a Damson, but differing in shape.

TABLE IV

SUMMARY OF ARTIFICIAL INOCULATIONS ON *Prunus cerasifera.* CONCLUDED NOVEMBER, 1914

Prunus cerasifera, Arnold Arboretum Type

Experiment Serial No.	Date	No. of Inoculations	Positive Inocuia-tions	Size of Gallsin Inches
595	6/20/14	10	10	$\frac{1}{4}$–$\frac{1}{2}$
646	6/29/14	10	8	$\frac{1}{16}$–$\frac{3}{16}$
709	7 6/14	10	9	$\frac{1}{16}$–$\frac{1}{8}$
776	7/13/14	10	8	$\frac{1}{16}$–$\frac{1}{4}$
22	7/20/14	10	5	$\frac{1}{16}$
902	7/27/14	10	8	$\frac{1}{8}$–$\frac{1}{4}$
963	8/ 3/14	Check	0	
A 1	8/10/14	10	4	$\frac{1}{4}$–$\frac{1}{8}$
*A*111	8/15/14	10	2	$\frac{1}{16}$
*A*149	8/17/14	10	4	$\frac{1}{16}$
*A*225	8/24/14	10	10	$\frac{1}{16}$–$\frac{1}{4}$
*A*253	8/31/14	10	2	$\frac{1}{16}$
		110	70	

Prunus cerasifera **(large four year old seedling).**

Experiment Serial No.	Date	No. of Inoculations	Positive Inocula-tions	Size of Galls in Inches
506	5/ 5/14	10	10	$1\frac{1}{4}$–$1\frac{1}{2}$
538	5/25/14	10	10	$\frac{1}{4}$–$\frac{1}{2}$
566	6/18/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
62	6/29/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
649	6/29/14	10	10	$\frac{3}{4}$–1
686	7/ 6/14	10	10	$\frac{1}{4}$–$\frac{1}{2}$
751	7/13/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
821	7/20/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
901	7/27/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
962	8/ 3/14	Check	0	
A 9	8/ 4/14	10	10	$\frac{1}{2}$–$\frac{3}{4}$
A 40	8/10/14	10	10	$\frac{1}{4}$–$1\frac{1}{2}$
*A*110	8/15/14	10	10	$\frac{1}{2}$–$\frac{5}{8}$
*A*148	8/17/14	1	10	$\frac{1}{4}$–$\frac{1}{2}$
*A*214	8/24/14	10	10	$\frac{1}{4}$–$\frac{1}{2}$
*A*251	8/31/14	10	10	
		150	150	

growing as rapidly, or, judging from their shrub-like growth, because they are of a different type possibly nearer to the wild type than are those commonly imported from France by nurserymen.

The variety known as Golden Beauty, *P. hortulana,* has thus far shown more marked resistance than other varieties of the species thus far tested. It is interesting to remember that this variety is supposed to have originated in western Texas some-

what out of the natural range of the species. *P. hortulana* and *P. americana* are used as a stock for the native plums in the middle west and east. *P. hortulana* does not sucker, fruits abundantly and has a number of excellent qualities that would recom-

TABLE V

SUMMARY OF ARTIFICIAL INOCULATIONS ON GOLDEN BEAUTY, *Prunus hortulana*. CONCLUDED NOVEMBER, 1914

Experiment Serial No.	Date	No. of Inoculations	Positive Inoculations	Size of Galls in Inches
600	6/20/14	10	7	1/16
651	6/29/14	10	3	1/16 very small
714	7/ 6/14	10	5	1/16–1/8
780	7/13/14	10	0	
826	7/20/14	10	4	1/16
906	7/27/14	10	1	1/16
967	8/ 3/14	Check	0	
A 11	8/ 4/14	10	3	1/8
A 44	8/10/14	10		
*A*114	8/15/14	10	1	1/16
*A*152	8/17/14	0	1	1/16
*A*250	8/29/14	10	0	
		110	25	

mend it as a stock. Further experiments with varieties of this species are being made and its adaptability to various of our stone fruits carefully studied.

TABLE VI

SUMMARY OF ARTIFICIAL INOCULATIONS ON *Prunus pumila* LINN. CONCLUDED NOVEMBER 15, 1914

Experiment Serial No.	Date	No. of Inoculations	Positive Inoculations
580	6/20/14	10	0
635	6/29/1	10	0
766	7/13/14	10	0
849	7/20/14	10	0
88	7/27/14	10	0
9 4	8/ 3/14	Check	0
A 8	8/ 4/14	10	0
A 34	8/10/14	10	0
*A*106	8/15/14	10	0
*A*144	8/17/14	10	0
*A*220	8/24/14	10	0
*A*254	8/31/14	10	0
		110	

Further inoculations of *P. pumila* (thirteen experiments of ten inoculations each or 130) made during the present year, 1915,

in vigorous growing seedlings, gave negative results, agreeing with the results of the two previous years. The experiments thus far conducted show that the species is entirely resistant to artificial inoculations. *P. Besseyi*, closely related to *P. pumila*, also shows considerable resistance.

The two other species of *Prunus* referred to as being resistant are *P. ilicifolia* and *P. Caroliniana*. They are evergreens and are not now considered as strictly belonging to the genus *Prunus*. They do not readily unite by grafting or budding with varieties of the stone fruits. *P. pumila* is a shrub and while this stock readily unites with many of the varieties of the stone fruits it probably would dwarf the tree more or less and might sprout. It is, however, readily grown from pits or cuttings.

TABLE VII

SUMMARY OF ARTIFICIAL INOCULATIONS ON *Prunus*. CONCLUDED OCTOBER 31, 1913

Species	Variety	No. of Inoculations	Positive Inoculations	Per Cent.	Size of Galls in Inches
P. domestica.....	German prune	75	2	2⅔	1/16–⅛
P. domestica.....	German prune	50	5	10	1/10–⅛
P. domestica.....	Italian prune	127	17	13	1/16–¼
P. domestica.....	Green gage	17	4	23	1/16–⅜
P. insititia.....	Damson	138	51	37	¼–¾
P. domestica.....	Duane	37	15	40	
P. persica	Elberta	61	57	93	½–2½
P. triflora × *P. Simonii*.....	Wickson (hybrid)	56	64	98	½–1
P. triflora.....	Burbank	31	31	100	¼–½
P. cerasifera.....	Myrobolan	8	8	100	½–1

In each of the inoculation experiments, ten punctures were made, hence the number of inoculations divided by ten will give the number of separate experiments made with the various hosts.

In the two tables, VII and VIII, where the inoculations of the years 1913 and 1914 have been summarized, there is a reasonable degree of consistency between the percentages shown for the different hosts. Other varieties of *P. persica* possibly should be further tested, although such varieties as Elberta, Saucer or Peento, Salway, Lovell and Muir seedlings have not shown any marked resistance.

Any of the stock as listed in Tables VII and VIII that show less than 50 per cent. infection are more or less promising, for in the experimental work with artificial inoculations from virulent

TABLE VIII

SUMMARY OF INOCULATIONS MADE ON *Prunus*. CONCLUDED NOVEMBER 15, 1914

Species	Variety	No. of Inoculations	Positive Inoculations	Per Cent. of Galls	Size of Galls in Inches
P. pumila	2 varieties, Arnold Arboretum	110	0	0	
P. domestica	Italian prune	140	10	7	1/16–1/8
P. cerasifera	*P. planteriensis*	40	3	7½	1/16–1/8
P. domestica	German prune	240	24	10	1/16–1/2
P. insititia	Damson	120	13	10	1/8–1/2
P. Besseyi	———	50	5	10	1/8–1/2
P. hortulana	Golden Beauty	110	25	22	1/16–1/8
P. amygdalus[4]	Bitter almond	100	22	25	1/8–3/8
P. domestica	Reine Claude (green gage)	90	25	26	1/16–1/2
P. Armeniaca	Mikado	40	11	27	1/16–3/16
P. angustifolia	Watsoni	50	15	30	1/16–1/8
P. maritima	Arnold Arboretum	140	48	34	1/16–1/8
P. dasycarpa	Arnold Arboretum	130	55	42	1/16–1/2
P. Mitis	Arnold Arboretum	60	32	53	1/16–1/8
P. cerasifera	Arnold Arboretum	110	70	63	1/16–1/4
P. Munsoniana	Arnold Arboretum	70	48	68	1/8–3/16
P. Munsoniana	Arkansas	90	70	77	1/16–3/8
P. Americana	Arnold Arboretum	100	83	83	1/16–1/2
P. hortulana	Arnold Arboretum	130	108	83	1/8–3/4
P. insititia	Pendula	90	77	85	1/16–3/4
P. davidiana	———	110	96	88	1/8–3/4
Schinus Molle	Pepper tree	110	97	88	1/4–1½
P. triflora	Burbank	120	109	90	1/4–1½
P. nigra	Arnold Arboretum	60	56	90	1/4–3/8
P. orthosepala	Arnold Arboretum	80	72	90	1/16–1/8
P. Mume	Arnold Arboretum	100	91	91	1/4–1/2
P. Munsoniana	Pits Arnold	140	130	92	1/16–1½
P. cerasifera	*P. divaricata*	100	94	94	1/8–3/4
P. Persica	Elberta	130	122	94	1/4–3/4
P. Armenica	Royal apricot	120	117	97	1/4–1
P. triflora	Arnold Arboretum	140	137	97	1/8–3/4
P. Munsoniana	El Paso	100	97	97	1/4–1/2
P. cerasifera	Sprouts	120	117	97	1/4–1½
P. triflora × *P. Simonii*	Wickson (hybrid)	140	138	98	1/8–3/4
P. cerasifera	Tree	150	150	100	1/4–1½
P. monticola	Arizona Experiment Sta.	40	40	100	1/2–1½
P. Simonii	Arnold Arboretum	130	130	100	1/4–1½

cultures, the different stocks were subject to a more severe test than obtains under the usual field conditions. The more promising of these, the first seven in Table VIII, with the exception of the damson, have already been considered after the various table in which the results were summarized. The damson has often

[5] *Prunus Amygdalus* was not growing well during the last part of the season and no infections showed after that of July 6. Before this time a large percentage of the inoculations were positive. This will be repeated another year.

been used as a stock, but is not popular on the Pacific coast because of its slow growth in the nursery and the difficulty of working it with many of the stone fruits. Duane, *P. domestica*, Table VII, is being used to some extent as a stock in California and shows resistance to gall in old vineyard land where it has been grown for six years. It makes as large a tree as the popular Myrobolan stock. The seedlings are grown from suckers, which give a root likely to sucker. Reine Claude (green gage) variety has shown resistance and would without doubt be a good stock for the domestic type of plums. *P. Armeniaca*, variety Mikado, is an apricot that differs somewhat from the one commonly grown in California. It should be tested out experimentally as a stock for apricots to replace the susceptible one now being used.

The almond, from field observations, is one of our most susceptible stock and this is fully confirmed by the following inoculation experiments: Fourteen different varieties of almond seedlings not summarized in the following tables were inoculated in April of 1913 at the University Farm at Davis, California. These in all cases showed a high percentage of infection. So far the peaches and almonds have shown only slight resistance.

It will be noted that our most popular stocks as Myrobolan, peach, apricot and almond are very susceptible, which only goes to confirm field observations that the stock used for the stone fruits are very susceptible to crown gall.

The work so far conducted shows that seedlings of the German and Italian prunes might be promising stock for certain of the stone fruits, probably those of the *domestica* type. However, no definite recommendations can be given, as the work is now only in its preliminary stages.

SHORTER ARTICLES AND DISCUSSION

EUROPEAN FOSSIL FISH-SCALES

In European Cretaceous deposits fish-scales have been found at various times, and occasionally have been named and described by paleontologists. Dr. A. S. Woodward, in his great "Catalogue of the Fossil Fishes," has carefully and accurately listed all the names so given, but has made little or no attempt to examine the records critically, assuming that they were valueless, or nearly so. More recent work on fish-scales brings out the fact that these materials are of great value for the understanding of Mesozoic fish life, but unfortunately they have been described with little knowledge of their significant characters. An important pioneer work was that of Geinitz,[1] describing scales from the Turonian of Saxony. Geinitz realized that it was necessary to make comparisons with scales of recent fishes, and gave a plate of "Schuppen von lebenden Fischen," but unfortunately chose species which had little relationship, for the most part, with the fossils studied. The three plates of fossil scales appear to have been very carefully drawn, and from them it is possible to gather a number of facts not brought out in the text. *Cyclolepis agassizi* appears to be Salmonoid, agreeing quite well with the modern *Salmo*. *Aspidolepis steinlai* is like the scales of living Stromateidæ, as *Poronotus*. *Osmeroides divaricatus* evidently has nothing to do with the genus to which it is assigned, but is of characteristic Albulid type. *Osmeroides lewesiensis* (Mantell), as determined by Geinitz, consists in the main of scales agreeing with those of the remarkable living genus *Pterothrissus*. The genus *Cladocyclus* presents some serious difficulties. The type is a Brazilian species (*C. gardneri* Agassiz) from the Upper Cretaceous of the Province of Ceará. I am indebted to Dr. D. S. Jordan for material referred to this species, and it appears that the large scales have extremely fine circuli, while those of the lateral line possess branching canals of the same general type as those of the living S. American genus *Hydrolycus*.[2] It does not

[1] "Die Fossilen Fischschuppen aus dem Plänerkalke in Strehlen," 1868.

[2] These canals are in the *apical*, not the basal field, as erroneously stated by me in *Annals Carnegie Museum*, IX, p. 110.

seem certain that this is the true *Cladocyclus* of Agassiz; it may represent a new genus ancestral to the Neotropical Characoid fishes. European "*Cladocyclus*" is in any event surely distinct from the Brazilian. The *C. strehlensis* of Geinitz includes scales approaching those of *Potamalosa*, but the species is founded in the main on an entirely different type, which is evidently close to the English *C. lewesiensis*. It is a question what generic name should be used for the *lewesiensis-strehlensis* type, which must be removed from *Cladocyclus*. It was formerly included in *Hypsodon* Agassiz, but that generic name appears to belong properly to the fishes usually called *Portheus*, the type being *lewesiensis* Mantell = *mantelli* Newton. The name *lewesiensis* also belongs strictly to the *Portheus*, and the English so-called *Cladocyclus*, if distinct from the Geinitz species, seems to need a new name. These matters will be taken up more fully elsewhere at a later date. *Beryx ornatus* of Geinitz, properly called *Hoplopteryx lewesiensis* (Mantell), appears to be a primitive Berycoid type, having scales such as might be expected in an ancestor of the modern Berycidæ. *Hemilampronites steinlai* Geinitz consists of scales differing little from the living *Hyporhamphus*. The scales figured by Geinitz as those of *Macropoma mantelli* Agassiz[3] have no resemblance to that species; from the fine transverse circuli, basal radii, and apical teeth like those of *Pomacanthus*, they appear to belong to some Teleost more or less related to the Berycoids. Thus we find that although Geinitz knew little about the affinities of his scales, they had excellent characters, reminding us in certain cases of modern genera, and indicating the great antiquity and constancy of peculiarities of scale structure. In 1878 Anton Fritsch[4] undertook to describe the fish-scales of the Upper Cretaceous of Bohemia, and believed that he had a number of the species of Geinitz. His *Cladocyclus strehlensis* and *Cyclolepis agassizi* are perhaps correct, but the others are evidently different from the Geinitzian forms. His *Macropoma speciosum* Reuss is a genuine species of that genus, with quite characteristic scales. His *Macropoma forte*, on the other hand, appears to be a *Cœlacanthus*. His *Osmeroides lewesiensis* has regular transverse circuli between the radii, instead of the minute tubercles (markings like the surface of a strawberry) of the Geinitz scales

[3] *Macropoma mantelli* should be called *Macropoma lewesiensis* (Mantell), based on the *Amia(?) lewesiensis* of Mantell.

[4] "Die Reptilien und Fische der böhmischen Kreideformation."

and of *Pterothrissus*. His *O. divaricatus* is wholly distinct from the scale described by Geinitz under that name, and has not the Albulid characters. The *Beryx ornatus* scales are printed upside down, and the artist has added ctenoid structures (small teeth) above, on what is really the basal margin. In 1874 T. C. Winkler published a paper[5] in which he described two species from the scales. His *Osmeroides belgicus* appears to be congeneric with the *Osmeroides lewesiensis* as understood by Fritsch. His *Cycloides incisus*, supposed new genus and species, of which he says that he knows no fish, living or fossil, with such scales, is apparently worthless. It may not be a fish-scale. In America the fossil scales of Teleosts have received very little attention, but a large collection accumulated by the U. S. Geological Survey is now under review, and will undoubtedly yield much of value for the understanding of Mesozoic fishes, and at the same time throw light on the ancestry and relationships of modern families.

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UNIVERSITY OF COLORADO, BOULDER,
July 8, 1916

[5] "Mémoire sur quelques restes du Poissons du systéme heersien," *Arch. Mus. Teyler.*, IV.

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THE SELECTION PROBLEM[1]

RAYMOND PEARL

OF all the supporters of the doctrine of natural selection as the chief factor in organic evolution, August Weismann was preeminent. He stood shoulder to shoulder with Wallace in his entire willingness to attempt the explanation by selection of any biological phenomenon whatsoever, and he far outstripped the latter in the keenness and subtlety of his logical powers when an especially difficult bit of exegetic activity was called for. Both, it hardly needs saying, left Darwin far behind in the extent of their advocacy of the *Allmacht* of selection. Certain it is that if any one could speak authoritatively, and without suspicion of either hostility or doubt, about the selection theory, Weismann could. For that reason it seems desirable to take as the starting point of this discussion a statement made by that distinguished biologist so lately as seven years ago. At the Darwin Centenary meeting in Cambridge, Weismann, discussing the adequacy of the selection theory to explain the initial steps of evolutionary change, said:[2]

> To this question even one who, like myself, has been for many years a convinced adherent of the theory of selection, can only reply: "*We must assume so, but we can not prove it in any case.* It is not upon

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 109. This paper constitutes the address of the retiring President, read in abbreviated form at the dinner of the American Society of Naturalists in New York, December 29, 1916.

[2] "Darwin and Modern Science," Cambridge, 1909, p. 25. The italics are in the original.

demonstrative evidence that we rely when we champion the doctrine of selection as scientific truth; we base our argument on quite other grounds."

Even since 1909 a good deal of water has flowed under all our bridges, and particularly under the evolutionary ones. Among other changes in viewpoint there is evident a marked disinclination in science nowadays to regard as "scientific truth" anything which is not based upon demonstrative evidence. But it is also a fact, perhaps at first thought to be regarded as curious, in view of the opinion of Weismann which has been quoted, that there are here with us to-day those who assert, with great zeal and pertinacity, that in selection is to be found the chief cause of evolutionary change. These things being so, it has seemed that possibly it might be profitable to spend a little time upon the selection problem, trying to determine whether the case is any better now than Weismann conceived it to be seven years ago, from the viewpoint of tangible objective evidence. It is to be hoped that it is, for among working geneticists just now any theory which has to depend for its sole support upon its "interpretative value"[3] is sure to receive scant attention.

So then what I shall try to do is to review briefly some of the real evidence about the selection problem which has been accumulating since biologists turned definitely to the experimental study of evolution, and definitely away from the glorious, but on the whole unproductive, attempt to solve its problems by *a priori* reasoning. From such a review it is to be hoped that we may get some light as to the directions in which further research and new evidence are most urgently needed.

I. NATURAL SELECTION

In considering the whole selection problem we may well begin with an examination of the theory of natural selection. The mere fact of natural selection, in the sense solely and strictly of a process leading to the elimination of some individuals and the survival of others, is no

[3] Weismann, *loc. cit.*, p. 50.

longer questioned by any one who takes the trouble either to think or to observe living things. It is a process which goes on constantly and affects all organisms. In this sense it is no more than the resultant of the observed absence of individual, mundane immortality among living things. The fact that individuals die implies that those not yet dead are a selected lot, in at least one respect, namely survival.

This mere fact of elimination and survival is, however, not in itself particularly illuminating. The first question before us is whether such a process is capable of bringing about evolutionary changes of a progressive sort. Obviously it is capable of doing so, in theory at least, if we add two assumptions, or better rules according to which the Dance of Death is to be performed. The first of these rules is that the individuals alive at any time shall be different from those dead, in some other respects than that of survival merely. In other words, the elimination shall be selective. The second rule is that the survivors shall transmit to their progeny those differences which mark them off from the eliminated. The theory that these two rules are always and everywhere in operation, taken together with the observed fact that living creatures do die, *is* the Darwinian theory of natural selection as a factor in organic evolution. If the premise be granted that the game of survival is in fact played by these rules, the conclusion is then logically irresistible that evolutionary progress is bound to occur in the direction of those differences which distinguish the survivors.

Here many have been content to let the matter rest. In the minds of an astonishingly large number of people, which number includes some rather great names in the world of science, it is precisely the same thing to show that something logically must be so, as it is to show that it is so. If the formal rules of logic are satisfied, truth seems to them to be thereby established. No further evidence is demanded. As every one knows, this attitude led practically to the intellectual bankruptcy of the whole

evolution theory in the late nineties, from which it was rescued only by the active movement towards an objective, experimental accumulation of facts about the subject. But the danger which lurks in formal logic is always threatening the progress of science. In the field of science in which we are interested the most recent conspicuous example of it is found in the vicious attacks on Mendelism, which upon analysis can be seen to have their only basis in a formally logical, so-called "proof" that it can not be true. The danger is so insidious, and takes such diverse forms, that one feels justified in quoting a brief statement made by Professor F. C. S. Schiller,[4] which might well, in sufficiently large type, be hung upon the wall of every biological laboratory, as a constant reminder that the foundations of scientific truth lie in experiment and observation, not in logic. Schiller says:

> The proof that any logic, which declines to consider the question of the real truth of the reasonings it attempts to deal with, necessarily condemns itself to utter formality is easily given, and very instructive. It is a formal characteristic of *every* assertion that it *claims truth*, absolutely and without reservation or suggestion of fallibility. Hence it follows both if (*a*) the question of the actual value of this claim is ruled out of order, and if (*b*) the assertion is accepted at its own estimation, that the distinction between true and false must, in fact though not in name, disappear from Logic. For all assertions will be held true because they formally claim truth; because none profess to be false, error no longer exists—for Logic. Thus the logical form of an assertion affords no means of deciding upon the real value of its claim to truth, and hence any logic which restricts itself to the study of this form inevitably accepts a truth-claim as the equivalent of real truth. It is like a bank which does not distinguish between promises to pay and hard cash.

Then clearly the question to which we want an answer is not whether natural selection *can* cause evolutionary changes, but rather whether it *does* cause such changes in any significant degree or extent. In other words, we shall prefer the "hard cash" of objective experimental evidence to any logical "promise to pay," however tight and compulsive its reasoning.

[4] Schiller, F. C. S., "Formal Logic. A Scientific and Social Problem." London, 1912, pp. 6–7.

The tale here is not a long one. Indeed it is surprisingly brief, considering the mass of literature which the theory of natural selection in its more formally logical aspects has engendered. We have first the pioneer work of Weldon[5] with *Carcinus*, in which a selective elimination of individuals different physically from the survivors was first demonstrated numerically, the eliminating environmental factor being the silt in the water. This was followed by a number of investigations of a more or less similar character, notably those of Poulton and Sanders[6] with *Vanessa*, and of di Cesnola[7] with *Mantis*, in which different colored forms of these insects were exposed to elimination by natural enemies, chiefly birds, with the result that there was found to be some relation between the chances of elimination and the degree to which the insect matched its background. Bumpus[8] studied surviving and eliminated English sparrows after a severe winter storm. Crampton[9] measured the surviving and eliminated pupæ of *Philosamia*, the elimination having been produced by wholly natural causes. Davenport,[10] in a very small lot of chickens, found that those killed by crows were colored differently from those eliminated. Lutz[11] found in *Drosophila* some differences in type between survivors and eliminated. Harris[12] has shown that among seedling beans abnormal types perished more frequently than strictly normal types under the same field conditions. The same author[13] has also made extended

[5] Weldon, W. F. R., *Proc. Roy. Soc.*, Vol. XLVII, pp. 360–379, 1894. Also see Brit. Assoc. Rept., Bristol (1898), pp. 887–902, 1899.

[6] Poulton and Sanders, Rept. Brit. Assoc. (Bristol), pp. 906–909, 1899.

[7] di Cesnola, A. P., *Biometrika*, Vol. III, pp. 58–59, 1909.

[8] Bumpus, H. C., Biological Lectures, Woods Hole, 1908, pp. 209–226, Boston, 1899.

[9] Crampton, H. E., *Biometrika*, Vol. III, pp. 113–130, 1904.

[10] Davenport, C. B., *Nature*, Vol. LXXVIII, p. 101, 1908.

[11] Lutz, F. E., *Bulletin Amer. Mus. Nat. Hist.*, Vol. XXIV, pp. 605–624, 1915.

[12] Harris, J. A., *Science*, N. S., Vol. XXXVI, pp. 713–715, 1912.

[13] Harris, J. A., *Science*, N. S., Vol. XXXII, pp. 519–528, 1910. *Pop. Sci. Monthly*, Vol. LXXVIII, pp. 521–538, 1912, and numerous other papers in *Biometrika*, AMER. NAT. and elsewhere.

researches on the elimination of organs in a series of different plants.

The critical value of these different investigations is not in every case equal. Some are distinctly fragmentary, and in others the differences between eliminated and surviving are so small as to be of extremely doubtful significance. If one examines critically the actual biometric constants in the more extended of these studies (*e. g.*, Crampton's and Bumpus's as analyzed by Harris[14]) he can not but be impressed with the doubtfulness of many of the differences. However, if we take all these researches at their face value, and give all the benefit of the doubt to the weak, then they agree in indicating that the survivors are of somewhat different type physically than the eliminated.

But nearly as many investigations have been made which show that, on the whole, the survivors are not physically different from those naturally eliminated. Again the studies of Weldon[15] on *Clausilia* come first. Closely related to these is di Cesnola's[16] work on *Helix*. All three of these investigations agree in showing no significant difference in physical type between the general population before elimination and the selected survivors from that population after elimination. There was in *Helix* and in *Clausilia laminata* some reduction in variability, but even that failed in another species of *Clausilia*. Kellogg and Bell[17] were not able to find any evidence that survivors and eliminated were different in respect of either type or variability under natural conditions, in the case of bees, or of the lady-bird beetle *Hippodamia*. Pearl,[18] in a much more extended series of observations than those of Davenport, found no relation between the colors of chickens and their elimination by

[14] Harris, J. A., AMER. NAT., Vol. XLV, pp. 314–318. 1911.

[15] Weldon, W. F. R., *Biometrika*, Vol. I, pp. 109–124, 1901, and *Ibid.*, Vol. III, pp. 290–307, 1904.

[16] di Cesnola, A. P., *Biometrika*, Vol. V, pp. 387–399, 1907.

[17] Kellogg, V., and Bell, R. G., *Proc. Washington Acad. Sci.*, Vol. VI, pp. 203–332, 1904.

[18] Pearl, R., AMER. NAT., Vol. XLV, pp. 107–117, 1911.

natural enemies. This result, it may be said, has been confirmed in subsequent years. Reighard,[19] in one of the most beautiful experimental studies of natural selection which has ever been made, found that there was no relation between the colors of coral, reef fishes and their elimination by natural enemies.

While the researches which have been mentioned do not exhaust the literature, they are all for which time can be spared now, and they are fairly representative of the whole of the distinctly meager experimental and quantitative evidence regarding selective elimination. On the whole, the net result is not so clear-cut and outstanding as could be wished. If a scientific person came here from some other planet with an earnest desire to inform himself about selective elimination, of which he had not before known anything, and read all the available real evidence on the point, he would be sure to come to some such conclusion as this: that in some cases natural elimination is certainly in some degree selective, while in other cases it certainly is not; and in the most favorable cases of all the selection is apparently not very rigorous. Grossly teratological abnormalities are eliminated. But the smaller deviations from type, which in theory ought to furnish the basis of selection, appear upon quantitative study less generally and sharply determinative of survival than might reasonably have been expected theoretically. The case regarding this first element of the theory of natural selection certainly seems far less strong, under the critical eye of experiment and measurement, than those of us who were nourished on Weismann, Romanes, and their like, would have supposed possible twenty years ago. Still the writer has no desire to be controversial about the matter and if any one is disposed to draw the opposite conclusion from the facts he is entirely welcome to.

Let us now turn to the consideration of our second rule, which must be fully enforced if natural selection is to be an important factor in the causation of evolutionary

[19] Reighard, J., Carnegie Institution, Publication 103, pp. 257–325, 1908.

change. This, it will be recalled, was that the survivors must produce offspring which bear characters like those which had led to the survival. Or, to put the matter crudely, the survivors must transmit their characters to their offspring. In pre-Mendelian days this phase of the subject was always neatly and summarily disposed of by stating, as one of the *facts* on which the theory of natural selection rested, that "variations are inherited" or "like produces like." Times have changed. We are a great deal less certain about that particular brand of inheritance which the theory of natural selection demands than we were before any one had taken the trouble to make experiments on heredity. The essential difficulty lies here. The differences upon which natural selection directly operates are somatic differences, by hypothesis and in fact. Every worker in genetics has learned since the truly epoch-making researches of Johannsen[20] to be extremely cautious in assuming *a priori* that any particular somatic difference is so inherited.

The writer has lately been experimenting with a character which very well illustrates this point. A not infrequent variation of the single comb in poultry is the appearance, on one or both sides of the comb, of a small excrescence, known technically as a side-sprig. Individuals exhibiting this variation have been selected for breeding purposes. But, so far as the experiments have yet gone, it does not appear that the offspring of such animals are any more likely to exhibit the variation than are the offspring of any random sample of single-combed fowls. Now suppose, for a moment, that in a state of nature the possession of a side-sprig on the comb gave a bird a distinctly better chance for survival than did a plain single comb. Those lacking the variation would then by hypothesis tend to be eliminated, but there is not the smallest indication that there would result any progressive evolution towards a side-sprigged race.

Now one might go on and review a great accumulation

[20] Johannsen, W., "Ueber Erblichkeit in Populationen und in reinen Linien." Jena, 1903.

of evidence from the work of de Vries,[21] Dewar and Finn,[22] Bateson,[23] Lloyd,[24] and many others, showing the failure of the theory of natural selection to account satisfactorily for various observed happenings in evolution. It is not my purpose to do this. These facts are all familiar, and indeed have become commonplaces of biological literature. We may, however, with some chance of profit try to generalize all this evidence. If we do so, the writer believes that the conclusion will be reached that natural selection is no longer generally regarded as the primary, or perhaps even a major, factor in evolution because of three general groups of facts, each well established by the common observation of many biologists. The first of these large facts is that all organisms possess in varying, but usually very large, degree the power of personal, immediate, individual, somatic adaptation to the environment. In consequence of this power of personal adaptation the survival expectation of an individual is not generally and regularly a function of any static, single-valued relation between its somatic structure, habits, or physiology, on the one hand, and the impinging environmental stresses on the other hand. Yet such a relation is implicitly assumed in that part of the theory of natural selection which affirms a selective elimination on the basis of somatic characteristics. The second broad fact is that, even when selective elimination on the basis of somatic characteristics does occur, it does not follow generally and regularly that the somatic differences on which the selection acted will reappear in the progeny, or in short be inherited, actual experience having abundantly demonstrated that a very great many of such somatic differences are not inherited. The third large fact is that observation indicates that in many cases evolutionary changes have come about by relatively large, discontinuous steps, the

[21] de Vries, H., "The Mutation Theory." Chicago.

[22] Dewar, D., and Finn, F., "The Making of Species." London.

[23] Bateson, W., "Problems of Genetics." New Haven, 1913.

[24] Lloyd, R. E., "The Growth of Groups in the Animal Kingdom." London, 1912.

new form being not merely fully differentiated at its first appearance, but also fully able to survive.

Natural selection is, from the point of view of modern genetics, a somatic theory. It begins and ends in somatic differences. Except under the most unusual and rare of conditions natural selection can not possibly operate *directly* upon germ-cells. It must, from the very nature of the case, work only indirectly upon the germ through the soma. Now it is a historical fact that just so long as the study of heredity confined itself solely to the somatic results of the process, substantially no advance in knowledge was made. Only when it was clearly perceived that heredity is primarily and fundamentally a problem in the physiology of *germ cells*, and that the soma is, as some one has said, only the mechanism which the fertilized egg uses to produce another fertilized egg like itself, did we begin to make progress. Can we suppose that these considerations have no meaning or application in our attempts to solve the larger problem of organic evolution?

If natural selection only could act directly upon germ cells we should have a different story to tell. The writer has lately been experimenting[25] with an agent which acts with extraordinary precision and definiteness in a selective manner upon the germ cells, killing or inactivating the weak, and leaving only the strong and resistant to produce zygotes, and somata. This substance is alcohol. In the case at least of the domestic fowl the evolutionary effects produced by this substance are remarkable in their magnitude and definiteness. It is not possible here to go into details, but briefly it may be said that the first general result of the continued administration of ethyl or methyl alcohol, by the inhalation method, to the parents in poultry, is to diminish progressively the fertility. But the smaller number of offspring formed by the surviving germ cells, after selective elimination in the gonads has occurred, are in every measurable respect distinctly and markedly superior to the normal individuals of the races

[25] Pearl, R., *Proc. Amer. Phil. Soc.*, Vol. LV, pp. 243–258, 1916; *Proc. Nat. Acad. Sci.*, Vol. 2, pp. 380–384; *Ibid.*, Vol. 2, pp. 675–683, 1916; *Jour. Exper. Zool.*, Vol. 22, 1917.

from which they come. Here is *real* selection making *real* evolutionary progress, because its point of application is the germ and not the soma. That the same agent may produce an evolutionary change in the opposite direction in another organism, the guinea pig, as has been shown by Stockard[26] to be the case, seems to mean, from the point of view of the present discussion, nothing more than that the direction and amount of any evolutionary change is, fundamentally, a function of two variables, the organism and the environment. If the same environmental stress produced the same evolutionary effect upon all organisms, then it would follow that all organisms in the same environment must necessarily be alike at the end of the process, which is, of course, not the case.

II. The Experience of Practical Breeders

Let us at this point leave our discussion of natural selection and turn to the other great aspect of the problem, artificial selection. Here we shall tread on surer ground, first, because it is where the great bulk of the experimental work on the selection problem has been concentrated, and second, because the considerable mass of reliable historical material about the origin and improvement of domestic animals and plants becomes available as a source of pertinent and critical evidence on the problem. At the outstart it may be recalled that it was on the supposed results of artificial selection, as set forth in the experience of practical breeders, that Darwin chiefly relied for objective evidence in favor of natural selection.

In general this evidence has been accepted very uncritically by followers of Darwin. This is not strange in view of the fact that there have been, and are now, relatively few trained biologists who know anything at first hand about the practical breeding of animals for the show ring, advanced registry test, or any other purpose which involves necessarily the production of élite specimens which shall rank measurably with the best of the breed.

[26] Cf. Stockard, C. R., and Papanicolaou, G., Amer. Nat., Vol. 50, pp. 65–88, 144–177, 1916.

This fact has led to some entirely unwarranted inclusions in the technical literature of biology. Statements in the agricultural press which were intended by their breeder-authors merely as harmless generalities on a subject about which they had not the slightest intention of being specific, have been accorded, by the laboratory evolutionist, the dignity and authority of detailed reports of actual breeding operations, and cited as valuable evidence on the problem of evolution.

This confusion has played particular havoc in discussions of the selection problem because of the general and usually quite irresponsible use of the term "selection" by practical breeders. In the literature of live stock breeding the word "selection" has been, and is being used to-day, to designate, upon occasion, every known kind of breeding operation. To illustrate: a fancier who bred a new variety of poultry started with a mongrel male bird which happened to possess just the combination of characters which he wanted in his new breed, as the result of a previous series of indiscriminate crossings. This male was crossed with a female of a well established breed which possessed some of the desired characters. The daughters from this mating were back-crossed to their sire, the original male bird, and so in turn were his granddaughters. The granddaughters' progeny constituted the new breed, full blown and breeding tolerably true. This was an entirely legitimate, and indeed usual, way of making a new breed. But the point lies in the fact that the breeder who did all this always refers publicly to the series of matings which has just been described as "this process of *selection*"!

Since Darwin selection has been a word to conjure with amongst the practical breeders. In most cases without any comprehension whatever of the exact technical sense in which the term was originally used breeders have taken the word itself as a sort of fetish. Darwin[27] himself speaks of artificial selection as "the accumulation in one direction, during successive generations, of differences

[27] Darwin, C., "Origin of Species," Chap. I, p. 26.

absolutely inappreciable by an uneducated eye.'' Men in whose breeding operations selection in this sense demonstrably plays no significant part whatever, attribute to its magic power all improvement in their animals and plants. This is harmless so long as one understands that the ''selection'' is verbal and not biological. But unfortunately it has not always been so understood. Consequently we find the literature of evolution cluttered with a lot of utterly preposterous statements about domestic animals and plants, masquerading as valid evidence for the selection doctrine.

So fixed in the minds of most biologists not acquainted with agricultural matters at first hand is the idea that the vast majority of improved varieties of plants and animals owe their origin, or their improvement, or both, to cumulative selection of slight differences, that it appears desirable to review briefly a few of the actual facts. One can not hope to do more than touch here and there in a great body of evidence, but at least representative cases may serve to indicate the general tenor of the whole.

We may consider first the cultivated grape, of which there are over 1,300 recognized varieties grown in this country. Hedrick[28] and his assistants have made a very careful and systematic study of the origin of these forms. After discussing the early history of the vine in this country he says (p. 52):

> We have found that the wild grapes of the country, valued but uncultivated for two hundred years, became through mere transplanting from the woods into the vineyards . . . one of our most important fruits.

Again in commenting upon the growth of the grape-growing industry he says (p. 62):

> The results achieved seem all the greater when one considers that many of the best varieties now grown are the first, and scarcely any are further removed than the second generation from wild plants.

But we should not be content with these general statements. Let us examine specifically the history of a well-

[28] Hedrick, U. P., ''The Grapes of New York.'' Albany, 1908.

known and very excellent "improved" variety, the Concord. Of this grape Hedrick says (p. 219):

> The Concord is known by all. The most widely grown of the grapes of this continent, it also represents the dominant type of our native species and with its offspring, purebred and crossbred, furnishes seventy-five per cent. or more of the grapes of eastern America.

He speaks of "the preeminently meritorious character of Concord which has enabled it to take first place in American viticulture."

Now for the origin and history of this paragon.

> The seed of a wild grape was planted in the fall of 1843 by E. W. Bull of Concord, Massachusetts, from which fruit was born in 1849. The wild grape from which the seed came had been transplanted from beside a field fence to the garden in which there was at least another grape, the Catawba, and the wild vine was open to cross-pollination. One of these seedlings was named Concord and the variety was exhibited before the Massachusetts Horticultural Society in the fall of 1852. The new grape was introduced in the spring of 1854 by Hovey & Company, of Boston. From the time of its introduction the growth of this variety in popularity was phenomenal. In 1865 it was awarded a prize by the American Institute . . . as the best grape for cultivation.

But where in the history of this "best" American grape is the gradual accumulation of minute variations by selection? The story is of the same sort for all varieties of grapes about whose origin anything is known; either they were chance seedlings, or F_1 hybrids, or F_2 segregates, grown under good conditions.

The same general situation obtains in regard to the origin and improvement of other fruits besides the grape. One need only mention various cases briefly to recall them to mind. New and improved varieties of apples, plums, cherries, strawberries, etc., have originated either as chance seedlings, or as bud variations, or as hybrids. In their production selection, in the sense of the accumulation of minute favorable variations, has had no part.

In the case of many fruits the mere fact of domestication accounts for all the improvement over the wild type. A striking example of this is found in the case of the most recently domesticated wild plant, the blueberry. Mr. F.

V. Coville[29] succeeded in discovering some years ago the two essentials for the successful cultivation or domestication of the swamp blueberry (*Vaccinium corymbosum*). These were found to be (1) an acid soil, and (2) a root fungus that appears to supply the plant with nitrogen. When these essentials are supplied, and the plant brought under cultivation, a marked improvement in the size and quality of the berries at once occurs. Further improvement is being made by hybridization, and by seeking superior natural variations to be used in such hybridization and for asexual propagation. In his latest paper Coville makes this statement, the significance of which in the present connection will be noted.

> Seedling plants, even from the largest berried wild parents, produce small berries as often as large ones.

Selection, in the Darwinian sense, is playing no part in the improvement of the blueberry, so far as we can learn from the published records. The essential factors in the improvement are better conditions, hybridization, and the asexual propagation or superior natural variations.

Let us now turn to other sorts of plants. In 1910 my colleague, Dr. Frank M. Surface, originated a variety of oats, known as Maine 340, which is superior to any variety which we have been able to find in the market and test. It is now widely grown in Maine. For the conditions of soil and climate in that state it is certainly to be regarded as the most highly "improved" variety known. Its origin and history are fully known, indeed are originally recorded in the archives of this laboratory.[30] All of the thousands upon thousands of bushels of this Maine 340 oat which were grown this year are the lineal, unchanged descendants of one particular oat plant which Dr. Surface isolated in 1910. That original plant has simply been multiplied, by seed, year after year without selection of

[29] Coville, F. V., U. S. Dept. Agr. Bulletin 193, 1910; Circular 122, 1913; Bulletin 334, 1915.

[30] Cf. Surface, F. M., and Zinn, J., Me. Agr. Expt. Stat. Ann. Rept. for 1916, pp. 97–148.

any sort or kind, after the first isolation of the plant which originated the variety.

The commercial variety of oats which comes the nearest under our conditions to equalling Maine 340 in yield and other desirable qualities is one known as the Early Pearl. The history of this variety has been given by Surface and Barber,[31] who got it from the originator, Mr. R. L. Copeland. Mr. Copeland says:

> The first seed was obtained from a bunch growing by the roadside some twenty years ago, presumably from one seed. It was examined and showed such merit that it was cut and preserved for seed. Although the first seed was not secured by me personally, it soon after came into my possession. The oat seemed to possess excellent qualities and as it matured fairly early and had a pearly tint to the hull I gave it the name of Early Pearl.

Since the beginning the oat has simply been grown by Mr. Copeland unmixed with other sorts.

Here again that painstaking and laborious selection, by which the practical breeder is supposed to make the wonderfully improved and valuable varieties which we have, is conspicuous by its absence. And one must not for a moment suppose that this oat is not a wonderfully superior one. Any oat which will yield, year in and year out, good seasons and bad, not less than about 80 bushels to the acre, and at the same time possess a whole series of other desirable qualities, which are too technical to go into here, is in the front rank of the products of the breeder's art. We find it to be surpassed only by some of our own new varieties. It outranks all other commercial varieties under our conditions.

Many other examples of the same sort of histories of varietal origin and improvement might be given. But de Vries[32] has covered the ground thoroughly and we need not stay longer over the plant side. The writer wishes to make clear before leaving the subject the reason for calling attention to these well-known matters. It is to emphasize that the "experience of practical breeders"

[31] Surface, F. M., and Barber, C. W., Me. Agr. Expt. Stat. Ann. Rept. for 1915, pp. 137–192.

[32] de Vries, H., "Plant Breeding." Chicago, 1907.

shows that the principle of the gradual accumulation by continued selection of minute somatic variations has had no essential part in the origin or amelioration of certainly a great many of the best varieties of agricultural plants which we have to-day. The essential factors which have been involved in the production of our best fruits, grains, vegetables, flowers, etc., have been (1) the improved conditions of domestication, (2) mutations, leading at once to new and better forms, (3) hybridization, which by new combinations of characters and as a result of heterosis[33] has led to amelioration, and (4) the purification of previously mixed races or varieties by selective sorting. It is to the overwhelming importance of one or a combination of these factors that the "experience of breeders" points and not to Darwinian selection.

But what of the animal side? Here the true facts are much more difficult to get at; in part for reasons which have been developed earlier in this paper, and in part for the reason that the making of new breeds of domestic animals is no longer going on to any extent except in the smaller sorts such as poultry. As has been pointed out elsewhere,[34] this is primarily a result of the great development of the system of pedigree registration, which puts a ban on cross breeding in cattle, horses, etc.

So then let us take as our first example one from poultry breeding where unequivocal facts are available. In his "Organic Evolution" Metcalf[35] makes the following statement:

> The extent of the modification produced by artificial selection is very great in many cases. Notice the common domestic chickens, in which the different breeds differ from one another to such a degree that if they occurred in nature the several kinds would be referred not only to different species, but to different genera. Compare the slender "game" which most closely of all resembles the ancestral "jungle fowl," with the heavy "Brahma" or "Cochin-china," or with the long-tailed "Japanese" cocks, or with the little "bantam."

[33] East, E. M., and Hayes, H. K., U. S. Dept. Agr., Bur. Plant Industry, Bulletin 243, 1912.

[34] Pearl, R., "Modes of Research in Genetics." New York, 1915.

[35] Metcalf, M. M., "Organic Evolution." New York, 1904.

All these forms, according to Metcalf's idea, have been produced by selection from the jungle fowl. For a number of years past the writer has been interested in collecting evidence regarding the making of new races and varieties of bantams, for the purpose of seeing what part selection, in the Darwinian sense, probably plays in the matter. The problem was given enhanced interest and significance by the appearance of the important paper by Punnett and Bailey,[36] which showed that in respect of body weight bantams are differentiated from large fowls by at least three genetic factors, and that in a cross between a bantam breed (Seabright) and a larger breed (Hamburg) the inheritance of body weight is typically Mendelian. This latter result the writer had found to be true, using different breeds from those employed by the English workers, but had not worked out in detail the exact mechanism of the inheritance. New varieties of bantams are all the time being produced and exhibited at poultry shows. Broadly speaking, it is nearly true that for every different variety of large fowl a corresponding bantam variety either has been produced or is sure to be soon. In view of this great activity in the making of new varieties it seemed that an excellent opportunity was offered to find out how the expert bantam fancier really turns the trick. So the writer has corresponded with bantam fanciers in all parts of the world and in this way has accumulated a large amount of interesting material.

One question was always asked, and always in the same form. This was:

> Do you know of any case in which a stable race, variety or breed of bantams, which bred true indefinitely in respect to bantam size of body, was originated or created *solely by selection* of small sized individuals of a *large* race, variety, or breed of fowls, without any crossing in of bantam blood? If so, please give a detailed account of the circumstances.

[36] Punnett, R. C., and Bailey, P. G., *Jour. Genetics,* Vol. IV, pp. 23–39, 1914.

The answers to this question were negative in every case except one. In that case the correspondent was unable to cite any specific instances illustrating his contention, but thought on general principles that it must be so. In other words, his belief in the selection theory, like that of Weismann, was based on wholly other grounds than demonstrative evidence. The general tenor of the answers to this question is well indicated by a statement of Mr. J. F. Entwisle,[37] who is probably the greatest authority on bantam breeds of poultry now living. He stated that it was thought by some that

> proper little bantams could be bred from large fowls without any admixture of bantam blood. . . . If such can be done, then our thirty-odd years' experience of bantam "manufacturing" counts for very little. We[38] have lived to see the manufacture of some forty varieties, and none without crossing, so far.

If time permitted, the citation of the detailed history of the making of several breeds of bantams would show very clearly that Darwinian selection plays an extremely minor and unimportant part in the process as it is actually performed. Large breeds of poultry show the same thing. Two of our most important breeds, the Wyandottes and the Orpingtons, are of comparatively recent origin. The facts as to their origin are well known and the essential biological factors concerned in both cases are the same, namely, hybridization as a start, followed by close inbreeding of desired segregating types. In other cases new varieties of poultry have appeared as sudden mutations by loss of factors. Such is the origin of the White Plymouth Rock and the White Cornish, for example.

III. Selection Experiments

We now come to the third class of evidence on the selection problem, namely, that afforded by controlled *ad hoc*

[37] Entwisle, J. F., *Poultry* (London), Vol. 30, p. 767, 1912.

[38] The "we" here is editorial. Mr. Entwisle is speaking of his own personal experience.

experimentation. Here the facts are so recent and so well known that a detailed review is unnecessary. I shall, therefore, only attempt to deal with them, for the most part, in a general way. Careful and critical selection experiments may be said in general to have given opposite results, according to whether the hereditary factors for the character which formed the basis of the selection were or were not positively known to be in a homozygous condition in all the individuals of the race experimented with. If the form used constituted a "pure line" in the strict sense of Johannsen's conception, so that every individual was surely known to be strictly homozygous for some state or condition of the character selected, and the mode of reproduction was such as automatically to retain and continue this condition, then the results of continued selection have in most cases been wholly negative so far as the production of any change in type is concerned. This is shown by the work of the Svälof Station[39] with various cereals, of Johannsen[40] with beans, of Jennings[41] with *Paramecium*, of Hanel[42] with *Hydra*, of Vilmorin[43] with wheat, of Ewing[44] with *Aphis*, of Surface and Pearl[45] with oats, of Fruwirth[46] with lentils, peas, soy-beans and lupines, of Lashley[47] with *Hydra*, of Agar[48] with *Simocephalus*, and of others.

[39] Cf. de Vries, H., *loc. cit. passim*, and Newman, L. H., "Plant Breeding in Scandinavia." Ottawa, 1912.

[40] Johannsen, W., "Ueber Erblichkeit in Populationen und in reinen Linien." Jena, 1902.

[41] Jennings, H. S., *Proc. Amer. Phil. Soc.*, Vol. 47, pp. 393–546, 1908; and AMER. NAT., Vol. 46, pp. 487–491, 1912.

[42] Hanel, E., *Jenaische Zeitschr.*, Bd. 43, pp. 321–373, 1908.

[43] Cf. Hagedoorn, A. L., and Hagedoorn, A. C., *Zeitschr. ind. Abst. Ver. Lehre.*, Bd. XI, pp. 145–183, 1914.

[44] Ewing, H. E., *Biol. Bul.*, Vol. 26, pp. 25–35, 1914; and *Ibid.*, Vol. 27, pp. 164–168; and *Ibid.*, Vol. 31, pp. 53–112, 1916.

[45] Surface, F. M., and Pearl, R., Me. Agr. Expt. Stat. Ann. Rept. for 1915, pp. 1–40.

[46] Fruwirth, C., *Zeitschr. f. Pflanzenzüchtung*, Bd. III, pp. 173–225, 1915.

[47] Lashley, K. S., *Jour. Exp. Zool.*, Vol. 19, pp. 157–210, 1915; and *Ibid.*, Vol. 20, pp. 19–26, 1916.

[48] Agar, W. E., *Phil. Trans. Roy. Soc.*, London, B, Vol. 205, pp. 421–489, 1914.

So far as the writer is aware the only important exceptions to the general rule exemplified by the above-cited researches are those obtained by Jennings[49] in *Difflugia,* and by his students, Middleton[50] with *Stylonychia,* and Stocking[51] with abnormalities in *Paramecium.* The facts in the case of these exceptions are beyond question. Just what their correct interpretation is does not seem to be so clear. Jennings himself (*loc. cit.,* p. 529) has expressed some doubt as to the significance of Miss Stocking's results so far as concerns their relation to normal reproduction. Morgan[52] has suggested that Jennings's *Difflugia* results may possibly be due to a sorting out of genetic diversities in the germ plasm, which came about from earlier conjugations, the material thus not representing a strictly homozygotic pure line. If this suggestion should prove to be valid the *Difflugia* work would fall at once into the same category as the cases of sorting out of pure lines from a mixed population by selection, with which the studies of Johannsen, de Vries and Jennings himself have made us familiar.

There is another point in connection with this extremely interesting and important investigation on *Difflugia* which seems to the writer, in the light of his own experience in breeding, of really extraordinary significance. One of the longest and most crucial selection experiments in the whole series was that for diverse numbers of spines in Family No. 326. During the first six periods after selection was begun in this experiment (p. 488) "no progress was made by selection." Then the basis of selection was changed. This change is described by Jennings in the following words (p. 489):

> After this time selection was based to a considerable extent on past performance. By this time many of the existent individuals had pro-

[49] Jennings, H. S., *Genetics,* Vol. 1, pp. 407–534, 1916.

[50] Middleton, A. R., *Jour. Exp. Zool.,* Vol. 19, pp. 451–503, 1915.

[51] Stocking, R., *Jour. Exp. Zool.,* Vol. 19, pp. 387–449, 1915.

[52] Morgan, T. H., "A Critique of the Theory of Evolution." Princeton, 1916.

duced several offspring. Where a parent of the low group had been found to bring forth high progeny, that parent was removed. Similarly, if a parent with a high number of spines is found to produce offspring with low numbers, this parent was removed. Thus in the low group we gradually tend to accumulate a set of individuals (1) which in the past have produced progeny with low numbers of spines; (2) whose ancestors for several generations back are individuals with low numbers of spines. In the high group the reverse conditions are fulfilled.

From the time this change in method was made until the end of the experiment, *selection produced a marked effect,* differentiating clearly high spine groups and low spine groups.

The significance of this result seems to me to lie in the fact that with the new method of selection described by Jennings the *ultimate basis of selection was changed from the soma to the germ.* Because after the change in method what *primarily* determined the selection of any particular individual for further reproduction was not its own spine number, but instead *its demonstrated ability to produce offspring with a particular spine number* (or nearly that number). A low spine parent was allowed to survive and reproduce not alone or primarily because it had few spines but only when in addition it was surely known to produce low spine progeny. This is indeed a different basis than that which merely selects individuals because of their somatic spine number and nothing else. It abruptly and completely transfers the selection from soma to germ. As has already been pointed out earlier in this paper (p. 5), there can be no question about the efficacy of selection which operates on a directly gametic rather than a somatic basis. This idea of making the basis of selection the ability to transmit to the progeny the desired quality is one which the writer has for a good many years strongly advocated as the only really useful or hopeful method in the practical breeding of higher animals. In his own work with poultry[53] it has been crowned with the highest practical success. Beyond

[53] Pearl, R., AMER. NAT., Vol. XLIX, pp. 306–317, 1915.

doubt or question the reason for the success is because by this *method we select directly the kind of gametes we want.* In ordinary Darwinian selection we select the *kind of somata* we want, and trust blindly that a wise providence has implanted in them the sort of gametes we *need* in order to get further somata like those we selected. And as in so many other troublesome affairs in this vale of tears, too often we find in the outcome that our trust has been misplaced!

It seems to me that whether Morgan's suggestion regarding the highly interesting and important results with *Difflugia* is true or not the *fact* dealt with in the preceding paragraph objectively parallels exactly the phenomena which one sees when he isolates pure lines from a mixed population (*e. g.*, in oats). He finds some individuals which are somatically what he wants but which fail to produce the sort of progeny he is looking for. These he discards. Others produce progeny which are like themselves. They transmit[54] their qualities and hence are retained.

Having considered the results of experiments on selection in pure lines we may turn to similar experiments with sexually reproducing organisms. Here the facts are, in the main, no less clear, but they are, on the whole, exactly opposite in their sense, at first sight at least. For in most of the experiments of this sort selection has been attended with an alteration of the type in the direction of the selection. This has been the case in the writer's[55] experiments on egg production, in the domestic fowl since 1908, in the work of Smith[56] and others with maize in

[54] Lest there should be any misunderstanding I may say that I am fully aware that the old idea of heredity as a direct and material transmission of personal qualities from generation to generation is wholly incorrect, out-of-date, and pedagogically pernicious. I find it, however, extremely convenient, saving of breath and good white paper, and, with this explanation, I hope permissible, to use "transmit" as a technical genetic term meaning "to possess gametes of such sort as will produce in the progeny."

[55] Pearl, R., AMER. NAT., Vol. XLIV, pp. 595–608, 1915.

[56] Smith, L. H., Ill. Agr. Expt. Stat. Bul. 128, 1908.

Illinois, in the work of Pearl and Surface[57] with maize, in MacDowell's[58] work on bristle number in *Drosophila*, in the work of Zeleny and Mattoon[59] with the bar eye of *Drosophila*, in the experiments of Castle and Phillips[60] with hooded rats, and in the work of some others. The difficulty with all these experiments is not in the facts but in their interpretation.

Before taking up this question of interpretation, however, it seems desirable to point out certain objective features which the results of these experiments have in common. The first is that they all occur (with the exception of the Jennings cases already discussed) in sexually reproducing organisms, not certainly known to be homozygotic with respect to all the factors which may be concerned in the production of the selected characters, and subject to a mixing of germ plasms in each generation. The second is that when any result at all follows selection in most if not all of the cases it comes quickly (*i. e.*, in a few generations), is relatively large in amount, and either no further change follows further selection or if it does occur it is again sudden and large in amount. This was true in the work with high producing lines of hens, of all work with maize by the ear-row method, and of MacDowell's with *Drosophila*, and also, as MacDowell[61] has so clearly demonstrated, in Castle's experiments with rats. A third point which strikes one is that in many of these successful cases of selection the basis of the selection has been fundamentally gametic, that of "progeny performance," rather than solely somatic. Individuals are selected for further multiplication which have demonstrated their ability to produce progeny bearing the desired somatic qualities. This was certainly the case in

[57] Pearl, R., and Surface, F. M., Maine Agr. Expt. Stat. Ann. Rept. for 1910, pp. 249–307.

[58] MacDowell, E. C., *Jour. Exp. Zool.*, Vol. 19, 1915.

[59] Zeleny, C., and Mattoon, E. W., *Jour. Exp. Zool.*, Vol. XIX, pp. 515–529, 1915.

[60] Castle, W. E., and Phillips, J. C., Carnegie Institution, Publ. 195, 1914.

[61] MacDowell, E. C., AMER. NAT., Vol. 50, pp. 719–742, 1916.

the Illinois corn work, as Surface[62] very clearly demonstrated. The writer[63] showed a number of years ago that it was the basis of success in selecting poultry for egg production. One strongly suspects it to be true in the other cases, though because the importance of the point has not been perceived, evidence in the published accounts is lacking on which to make any positive statement.

What is the correct interpretation of these favorable results? Two opposed opinions are held. Fortunately the heat of the controversy has been intense enough to produce some distillation and we at least have the issue very clearly and sharply defined. On the one hand it is held, because there has been an alteration of type in point of time coincident with successive selections, that selection on the basis of personal *somatic* qualities only, as such, in and of itself, has altered hereditary factors in the germ plasm. This view makes selection a cause of genetic variation,[64] a total reversal of the position held by Darwin and most of his followers. The opposing view is that selection can only be successful in altering the type when hereditary determiners to produce the desired somatic qualities are already present in the germ plasm. Selection, on this view, has nothing whatever to do with the causation of the variation, and is wholly powerless and without effect on the race unless either (*a*) the basis of the selection is directly gametic, by means of progeny performance test, or (*b*) the somatically selected individuals happen by good fortune to carry the necessary hereditary determiners in their germ plasm.

The opposition here really goes very far back. It is the world-old fight between heredity and environment, nature and nurture, germ and soma. One side believes first, that hereditary determiners or factors fluctuate reg-

[62] Surface, F. M., IV[e] Conf. internat. de Génétique, Paris, 1911, pp. 221–235.

[63] Pearl, R., AMER. NAT., Vol. XLV, pp. 321–345, 1911.

[64] Castle, W. E., *Sci. Mo.*, Vol. 2, p. 91, 1916, and Castle, W. E., and Phillips, J. C., Carnegie Inst. Publ. 195, p. 31.

ularly and frequently, if not indeed usually, and in high correlation with somatic characters; second, that mixing of germ plasms in fertilization alters hereditary determiners mutually and hence is, in and of itself, a cause of genetic variations, and, therefore, third, that a purely external agent, the continued selection of personal *somatic* qualities, will alter the germ plasm.[65]

The other side believes first, that the *germ plasm* is fundamental and remarkably conservative, basing this belief on such observations[66] on the one hand as those of Walcott that pre-Cambrian annelids, snails, crustaceans, and algæ were in many cases so like forms living to-day as to belong to the same genera, though a period of time variously estimated at from 60 to 200 million years has elapsed; and, on the other hand, those of Wheeler on ants enclosed in amber two million years ago but morphologically identical with forms living to-day. It believes, second, that when the germ plasm changes it does so as a result either of wholly internal physiological causes, or of very extraordinary environmental stresses acting directly upon the germ cells; third, that mixing of germ plasms, in and of itself, does not mutually alter hereditary determiners, basing this belief on the regularity, constancy and cleanness of typical Mendelian segregation; and fourth, that selection only acts as a mechanical sorter of existing diversities in the germ plasm and not as a cause of alteration in it.

The alternative views have been presented. In the present state of knowledge nothing is to be gained by mere assertions of opinion as to which more nearly represents the truth. But one may at least advance the view,

[65] In his last paper MacDowell has justly emphasized the scientific futility of defining and codifying the opponent's position in a controversy, so that one may the more neatly bowl him over. I am very sensible of the force of this point, and therefore have been at great pains in the preceding sentence to make only such statements as can be supported by verbatim quotations from the literature. Since, however, it seems to me equally important to keep the personal element out of scientific controversy, so far as may be, I do not make the specific references.

[66] Cited from Loeb, J., "The Organism as a Whole." New York, 1916.

to which the whole of this paper has been leading, and which one may hope will be heartily concurred in by both sides in the selection controversy, that the great outstanding need in research on the problem of evolution in general, and of selection in particular, *is more, and more searching, investigations as to the causes of genetic* (*factorial*) *variation*. That both sides realize this need, and are all the time bending more and more energies to its selection, is indeed cause for congratulation and augurs well for the future of that branch of biological science in which America has taken a leading place.

MENDELIAN FACTOR DIFFERENCES VERSUS REACTION SYSTEM CONTRASTS IN HEREDITY. II

T. H. GOODSPEED AND R. E. CLAUSEN

The literature on species crosses shows clearly that all gradations occur as regards fertility from complete sterility to what is apparently complete fertility. Recombinations may, therefore, in some cases, occur freely between species that appear to be distinct. Lotsy (1913) in particular has shown this to be true for a number of species crosses in *Antirrhinum* and doubtless such instances might be multiplied considerably. But even in these cases the behavior of the hybrid progeny in subsequent generations indicates that there is a possibility that some of the recombinations do not form functional reaction systems because of the discordant elements they possess. This is shown in the entirely new characteristics which a certain portion of the population may exhibit and in the peculiar ratios which are sometimes obtained in segregation. Such results only bear out more completely the conception that for any recombination of elements to be completely functional they must together form a harmonious reaction system. Even Detlefsen's (1915) results with the cavy species cross which gave in F_1 sterile males and fertile females may be brought into line with such a physiological conception perhaps better than by trying to account for it on the basis of any definite number of Mendelian factors. At least in the cavy cross the attempt to account for the results in this latter manner demonstrated that the number of factors concerned must be relatively large, and this is precisely what would be expected on the basis of recombinations involving whole choromosomes or sections of chromosomes carrying with them, perhaps, many discordant elements. If we recall the observation that no crossing-over occurs in the sex heterozygote (Morgan

et al, *l. c.*), presumably the male in this case, then it would be possible in the male cavy to secure only recombinations involving whole chromosomes, or in other words involving the building up of reaction systems with a large number of interchanged factors. In the female, however, these recombinations might include, in addition to those resulting from redistribution of whole chromosomes, cross-over gametes resulting from the exchange of sections of chromosomes. Such gametes might conceivably contain fewer discordant elements or they might be distributed in such a way as to distrub the reaction system thus formed less profoundly. Such data of course need first to be reexamined from this viewpoint before any definite conclusions can be reached, but the list of species hybrids which Detlefsen gives which result in sterile males and fertile females would seem to indicate that this is a phenomenon connected in some way with the observed lack of crossing-over in the sex-heterozygote already demonstrated in the work with *Drosophila* and the silkworm (Sturtevant, 1915). Until we know more about the fundamental basis of crossing-over and the factors affecting it, it is idle to speculate on such differences in behavior as are shown by males and females in the species crosses mentioned.

The insistent way in which the species hybrids between *Tabacum* and *sylvestris* point to the conception of the Mendelian reaction systems as units in themselves is of interest because of the broad and far reaching consequences which follow the application of such an idea. For if the nature of the progeny of these partially sterile hybrids as grown through several generations points to anything, it is that the abortive ovules and pollen grains represent a selective elimination of certain types of recombinations. Obviously, then, the presence of any considerable proportion of sterile ovules or pollen grains in plant material may be a consequence of hybridity and that of a rather profound type involving reaction systems which are more or less specific in their nature and in part incompatible with each other. The importance of this

fact has, perhaps, not been sufficiently emphasized in work involving material which displays such partial sterility. Obviously, however, it is impossible to regard ratios obtained from such material as of any significance, unless it be possible to demonstrate definitely the nature of such gametes as fail to function. It is, therefore, not strange that species hybrids as a class require a somewhat different sort of treatment from that applicable to intervarietal crosses involving relatively few factor differences.

In the extensive work which has been done with the various species and forms of *Œnothera* this influence of partial sterility has undoubtedly played an important part, but at the same time one which has not been clearly defined. Jeffrey (1914) in particular has sought to establish the hybrid nature of *O. Lamarckiana* on the basis of the high percentage of abortive pollen grains which are found quite generally in the genus, and Heribert-Nilsson (1912) has attempted to analyze the material from a Mendelian standpoint. These attempts have not, however, led to a consistent explanation of the results observed, although they have established certain peculiar conditions in *Œnothera* which practically preclude the application of a rigid Mendelian analysis to such a type of behavior. On the other hand, a critical examination of the phenomena displayed by the various forms of *Œnothera* clearly indicates that these belong to several distinct categories, and not to one as is very generally assumed in discussions bearing on this subject. In this brief discussion we propose to classify them roughly as follows:

1. Strict factor mutations arising from unknown causes but clearly referable to specific germinal changes in isolated loci in the hereditary system. These display a simple, consistent Mendelian behavior when tested with the forms from which they arise. They usually show relatively simple and definite character differences when compared with the parent forms rather than complex differences throughout.

2. Segregation phenomena of a complex type resulting

in the constant and continual production of a number of distinct forms which display for the most part a complicated behavior when tested with the parents from which they arise and with other forms. Such forms are often widely different from the parent forms in all or nearly all of their characters.

3. Chromosome duplications resulting in duplication of one or more or even all of the chromosomes to the extent of tetraploidy in some forms.

The first of these categories may be rigidly distinguished from the other two, both of which may be fundamentally expressions of some inherent condition in the "mutating" individual. The existence of this first type of mutation can scarcely be denied in the face of the extensive evidence accumulated both on the plant and on the animal side. In addition to this long series of past observations, we have now the extensive work of Morgan and his associates (*l. c.*) in which the origin of over a hundred such factor mutations has occurred under observation in *Drosophila* cultures. These investigations indicate clearly that such mutations are fundamentally dependent upon actual changes in the germinal substance. They are the loss-mutations of genetic literature, but with the abandonment of the presence and absence hypothesis such a classification, of course, loses its significance. Practically always only one locus is involved in such a change, and the new form displays a consistent alternative behavior when tested with the form from which it arose. Such mutations are usually recessive, although a few dominant ones have been secured. When obtained in pure culture they show no tendency to revert to the parent form, at least not more frequently than they tend to change in entirely different directions. They are relatively rare, they do not recur in any definite considerable ratio in any strain, and they are not clearly due to any specific cause. In *Œnothera, rubricalyx* appears clearly to be a dominant mutation of this type (Gates, 1914). There seems to be little occasion for confusing these strict

factor mutations with the more complex type of behavior included under the second and third categories.

When the second category is considered we are met with the task of harmonizing a large mass of rather confusing data, and it is perhaps true that this can not be done successfully at the present stage of our knowledge. There are not lacking, however, as many others have pointed out, a number of significant facts which are at least as logically explainable on the basis of hybridity as on assumptions of general germinal changes. If the results which have followed species crosses are to be extended to the type of behavior displayed by *Lamarckiana*, then it is clear that the numerical ratio in which segregation occurs is of no particular significance except in so far as its constancy indicates that it is due to a specific behavior in gametogenesis. Lotsy (1912) in particular has pointed out that in *Antirrhinum* species crosses, races may be secured which behave very much like some forms of *Œnothera* with respect to the segregation ratios obtained. The significant facts with which we have to deal are apparently, first that in *Œnothera* these "mutations" affect the sum total of the characters of the individuals, *i. e.*, they are dependent on complex germinal *differences* when compared with the parents, and second that these "mutations" continually recur within certain races in fairly constant ratios. These are facts which are just as simply explained on the basis of a complex type of segregation in which many of the systems formed contain discordant elements and therefore fail to develop as to refer them to actual change in germinal substance. Moreover, the former explanation harmonizes the results obtained with the simpler category of strictly Mendelian phenomena.

That this view of the *Œnothera* situation has something in its favor beyond the known general occurrence of partial sterility in this genus is shown by some of the results of hybridization between the various forms of *Œnothera*. The frequent appearance of F_1 populations consisting of distinct forms is usually taken to be an expression of

segregation in the gametic series of such forms. Beyond this the *Œnothera* phenomena display a remarkably orderly behavior, and, although complex in nature, such orderliness points strongly to some kind of definite segregation dependent upon the hereditary constitution of the forms involved rather than upon any change in germinal substance expressed in the gametic series. *Lamarckiana,* for instance, produces constantly a small percentage of *nanella* as well as a number of other forms. It is, therefore, necessary to assume merely that *nanella* gametes make up a small percentage of the gametic series in *Lamarckiana.* When two *nanella* gametes meet, a *nanella* individual is produced, and it breeds true, as might be expected. When, however, a *nanella* gamete meets a *Lamarckiana* gamete a *Lamarckiana* individual is produced, which need not necessarily differ in its behavior with respect to the production of *nanella* from other *Lamarckiana* races, since practically all of the combinations involving *nanella* elements would fail to develop, or in case some few of these combinations did develop, they might produce other characteristic forms unlike either *Lamarckiana* or *nanella.* This conception is in part borne out by the fact that *nanella* appears to differ from *Lamarckiana* not only in stature, but also in other characters, as is shown in hybridization phenomena involving this form. From this standpoint also, the occurrence of *nanella* in the progeny of a wide range of forms is perfectly intelligible. When *nanella,* which apparently really breeds true, is crossed back with *Lamarckiana* a small proportion of the progeny is of the *nanella* type and the rest are *Lamarckiana.* This proportion is apparently greater than that normally obtained from selfed *Lamarckiana,* and this is merely a consequence of the realization of the gametic ratio of *nanellas* in the *Lamarckiana* series as a phenotypic ratio. The true breeding of the forms thus resulting, to the extent that they breed true, is a natural consequence of the germinal constitution of such forms. On the other hand, when *rubrinervis,* which never produces *nanella* as a "mutant" (Gates, 1915), is crossed

with *nanella*, *nanella* does not appear in F_1, and in F_2 appears in a fairly definite ratio in the progeny of the *subrobusta* forms thus produced (de Vries, 1913), whereas the *Lamarckiana* forms produced breed true, after their usual fashion. Obviously this behavior is simply a consequence of the fact that *rubrinervis* does not produce a gametic series containing *nanella*, and, therefore, *nanella* can not appear in F_1. The impressively orderly behavior throughout in these hybridization phenomena is an eloquent testimony of the existence of a casual agency of a simpler nature than that called for under the hypothesis of actual, germinal changes.

Those phenomena included in the third category and dealing with chromosome duplication may be referred to the same type of irregular behavior in gametogenesis as is concerned in the production of the other "mutants." They are of interest most particularly from our standpoint with regard to the structural relations which they display in comparison with the other forms. Thus there is a series of *lata* forms dependent on the duplication of one chromosome in *Lamarckiana*. They are not identical within the series, but at least three or four are known which differ from one another in a characteristic fashion. This may well be dependent on the particular chromosome pair which is concerned in the duplication as Gates (1908) has suggested. According to the chromosome view of heredity this duplication has the effect of altering the proportions of the various elements in the reaction system, and naturally in a delicately balanced system such alteration results in a change in the somatogenic processes in a definite direction, the latter dependent upon which particular elements are increased. Since a whole chromosome with presumably a large set of factors is involved, it should follow that the entire set of characters of the plant would be affected, as appears actually to be the case. The important point in connection with these phenomena is the apparent consequence that phenotypic changes may be dependent merely on an alteration in the relative quantitative proportions of the

Mendelian units making up the reaction systems. Apparently this will account satisfactorily for the orderly resemblance of these chromosome "mutants" to each other and to the forms from which they arise. Conceivably the great body of data on which the mutation theory is based would, for the most part, find a simpler explanation along the lines thus indicated.

Summary

Summarizing briefly the content of this paper, the following facts have, on the experimental side, been presented with reference to a species hybrid:

1. *N. sylvestris* when crossed with various varieties of *N. Tabacum* gives F_1 hybrids which are replicas on a large scale of the particular *Tabacum* variety concerned in the cross.

2. The F_1 hybrids of *sylvestris* and *Tabacum* produce a small number of functional ovules which represent the *sylvestris* and *Tabacum* extremes of a recombination series, the great majority of the members of which fail to function because of mutual incompatibility of the elements of the two systems.

3. Back crosses with *sylvestris* give *sylvestris* and aberrant forms, and of the two the *sylvestris* alone are fertile and breed true. On the other hand, back crosses with *Tabacum* produce apparently only *Tabacum* forms of which some are completely fertile and continue to produce only *Tabacum* forms.

On the theoretical side the following conclusions have been drawn and their application indicated:

1. As a consequence of modern Mendelian developments, the Mendelian factors may be considered as making up a reaction system the elements of which exhibit more or less specific relations to one another.

2. Strictly Mendelian results are to be expected only when the contrast is between factor differences within a common Mendelian reaction system as is ordinarily the case in varietal hybrids.

3. When distinct reaction systems are involved, as in species crosses, the phenomena must be viewed in the light of a contrast between systems rather than between specific factor differences, and the results obtained will depend upon the degree of mutual compatibility displayed between the specific elements of the two systems.

4. Sterility in such cases depends upon non-specific incompatibility displayed between the elements of the systems involved, and the degree of this sterility depends upon the degree of such incompatibility rather than upon a certain number of factors concerned in the expression of such behavior.

5. The consequences of the application of such a conception to the complex type of behavior in *Œnothera* are pointed out, and the suggestion is specifically made that the type of behavior exhibited by *Lamarckiana* and its segregants in hybridization may be referred to such complex system interactions.

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SHORTER ARTICLES AND DISCUSSION

PIEBALD RATS AND MULTIPLE FACTORS

In the Naturalist for December, 1916, MacDowell has published an extended criticism of experiments in the modification of the hooded pattern of piebald rats by selection, in which my colleagues and I have been engaged for some years. This is not the first time that readers of the Naturalist have had their attention called to these experiments by similar adverse criticism and they are possibly quite weary of the subject. In so far as MacDowell merely offers in new form arguments which have already been presented by Muller and Pearl and answered by me, I shall make no reply. But as regards two points which may fairly be considered critical, one of which actually is so designated by MacDowell, I desire to present some evidence which I regard as conclusive but which MacDowell has not discussed, evidence possibly not accessible to many readers of this journal. MacDowell's criticism is based on the data presented in Publication 195 of the Carnegie Institution (Castle and Phillips, 1914), and in a brief paper in *The Scientific Monthly* (1916). Many additional data are given in Publication 241 of the Carnegie Institution, but this is not considered by MacDowell, although it was issued in September, 1916, as Paper No. 26 of the station with which he is connected, nearly two months in advance of his own paper. Had he considered carefully the evidence contained in this later publication, I am sure that he would have modified his criticism materially.

In 1914 Phillips and I offered two alternative explanations of the progressive changes observed under selection in the hooded pattern. These were (*a*) variability of the unit-character ("factor") hooded, and (*b*) multiple modifying factors affecting the hooded character. We found it difficult to decide between these two interpretations on the basis of evidence then available. For this hesitancy we were promptly taken to task by Muller, who championed the multiple factor interpretation now adopted also by MacDowell. MacDowell elaborates in much detail a dozen points which show compatibility between our observations and a multiple factor hypothesis, but without consid-

ering whether they are also compatible with the alternative hypothesis of a single varying factor. Modification on crossing, decreasing variability, regression, greater variability in F_2 than in F_1, effective return selection—these are all phenomena to be expected equally on either hypothesis. To cite them is no argument for one hypothesis rather than the other. This point has wholly escaped both Muller and MacDowell, who seem quite unable to conceive any but the single explanatory principle of multiple factors.

Putting aside these irrelevant arguments, there remain two points in MacDowell's discussion which require further consideration. They are the same two points which led us in 1914 to hesitate between the alternative interpretations which we considered, but on which we now have fuller evidence.

But before we go into this new evidence one or two minor points may be noted in which the accuracy of our generalizations is questioned. MacDowell has gone over our 1914 publication in great detail, devoting as many pages to its destructive criticism as we to its original exposition, and recalculating the statistical coefficients. It is gratifying to know that he has detected in these no serious errors, though his figures differ from ours slightly in some cases. Whether his calculations are more accurate than our twice-checked ones, I am unable to say without detailed reexamination of the data. As these are public property, the curious reader may satisfy himself on the point. I do not consider it necessary to go into the matter anew since the substantial correctness of our findings is not challenged.

MacDowell thinks that we did not sufficiently emphasize the decreasing variability (standard deviation) and the decreasing rate of divergence of the selected races, observed as the selection progressed. These to his mind imply that selection would sooner or later cease to be effective. In this opinion I can not concur, since in neither the plus nor the minus selection series has the standard deviation decreased by half, although sixteen successive selections had been made and the hooded character had been so modified as to be scarcely recognizable longer. Whether one considers the decrease in variability large or small depends principally upon how much importance he attaches to the values found for the first two generations of the experiment, when the numbers of individuals observed were still small and methods of grading them had not yet been fully standardized. Mac-

Dowell emphasizes the high variability of these early generations, few in individuals, and attaches importance to the *relatively* smaller variability of later generations. It seems to me fairer to compare the first half of the series with the second half. Concerning the point I have said (Publication 241, p. 172):

> The amount of the variation as measured by the standard deviation is less in the last half of the experiment than in the first half. It is also *steadier*, owing in part doubtless to the fact that the numbers are larger, and in part to a more stable genetic character of the selected races. But the genetic variability is plainly still large enough to permit further racial modification and there is no indication that it will cease until the hooded character has been completely selected out of existence, producing at one extreme of the series all-black rats, and at the other end of the series black-eyed white rats.

It should be noted that these conditions have already been approximated in individual cases.

THE NEW EVIDENCE

1. *The progeny of plus selected crossed with wild rats.* (Quoted without change from Publication 241, pp. 163–168.)

In 1914 Castle and Phillips published a report on breeding experiments with hooded rats, in which it was shown that the hooded color-pattern—itself a Mendelian recessive character in crosses with the entirely colored (or "self") coat of wild rats—is subject to quantitative variation, and that different quantitative conditions of the hooded pattern are heritable. (Compare fig. 36, plate 7.) It was also shown that by repeated selection of the more extreme variations in the hooded pattern (either plus or minus) it is possible gradually to modify the racial mean, mode and range as regards these fluctuations, without eliminating further fluctuation or greatly reducing its amount. We concluded that the unit-character, hooded color-pattern, is a quantitatively varying one, but were at that time unable to decide whether the observed variability was due simply and exclusively to variation in a single Mendelian unit-factor or partly to independent and subsidiary modifying Mendelian factors.

Since publication of the above I have been engaged in further experiments designed to show which of the alternative explanations is the correct one, and these are now sufficiently advanced to indicate definite conclusions. Previous experiments had shown that when a race of hooded rats, whose character has been modified by selection (either plus or minus), is crossed with wild rats, the extracted hooded animals obtained in F_2 as recessives show regression toward the mean condition

of the recessive race before selection began. This result suggested that the regression observed might be due to removal by the cross of modifying factors, which selection had accumulated in the hooded race. If this view was correct, it was thought that further crossing of the extracted hooded animals with the same wild race should result in *further* regression, and that if this further regression was *not* observed a different explanation must be sought for the regression already noted.

The entire experiment has accordingly been repeated from the beginning, with the same result as regards regression in the first F_2 generation, but with *no regression* of the same sort in a second F_2 containing twice-extracted hooded animals. So far from observing further regression as a result of the second cross with wild rats, we have unmistakable evidence that the movement of the mean, mode and range of the hooded character has been in the *reverse* direction. So the hypothesis of modifying factors to account for the regression and for the progressive changes observed under selection becomes untenable.

In repeating the experiment of crossing hooded rats of our selected races with wild rats, great care has been taken to employ as parents individuals of the greatest racial purity and to inbreed the offspring brother with sister, thus precluding the possibility of introducing modifying factors from other sources. In making the second set of crosses, the extracted individual has, wherever possible, been crossed with its own wild grandparent. In the few cases in which this was impossible, wild animals of the same stock have been used. This stock consisted of a colony of wild rats which invaded the basement of the Bussey Institution apparently from a near-by stable. Owing to faulty construction of the building they were able to breed in spots inaccessible to us, and it took many months of continuous and persistent trapping to secure their extermination. During this period we trapped a hundred or more of them, all typical Norway rats, colored all over, without even the white spot occasionally seen on the chest of wild rats. Two generations of rats from this wild stock have been reared in the laboratory, and all have this same self-colored condition.

The hooded animals used in the experiments to be reported on in this connection consisted of 4 individuals of the plus selected series, a male and 3 females, as follows:

TABLE 140

Individual.	Grade.[1]	Generation.
♀ 5513.....	$+4\frac{1}{4}$	10
♂ 6348.....	$+4$	10
♀ 6600.....	$+4\frac{1}{4}$	12
♀ 6955.....	$+4$	12

[1] See figure 35, plate 7 for significance of the grades.

Each of these animals was mated with a single wild mate, and their children were weaned directly into breeding cages containing a male and two or three females (brother and sisters). In the case of two matings, F_1 males of the same parentage were at the time lacking and males from a different cross were used. The results of such matings are tabulated by themselves and serve a useful purpose as controls. The F_1 animals all closely resembled their wild parents, but many of them had a white spot on the chest. They ranged from grade $+5\frac{1}{4}$ to $+6$ (self).

The F_2 animals are classified in table 141, where it appears that 73 of them were hooded and 219 non-hooded (*i. e.*, like F_1), an exact 1:3 ratio. More than half of this F_2 generation consists of the grand-

TABLE 141

Table 141 shows the classification of extracted hooded *first* F_2 young obtained from crossing hooded rats of the plus-selected series with wild rats.

Hooded grandparents.	Grade of hooded grandchildren.											Total hooded.	Total non-hooded.	Means of hooded.
	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4			
♀ 5513, $+4\frac{1}{4}$, gen. 10....	1	..	3	2	1	7	8	6	5	7	1	41	107	3.05
♂ 6348, +4, gen. 10.....	..	..	1	..	1	2	4	3	4	6	1	22	68	3.28
♀ 6955, +4, gen. 12.....	..	..	..	..	..	..	..	1	1	3	..	5	27	3.51
♀ 5513, $+4\frac{1}{4}$, and ♀ 6600, $+4\frac{1}{4}$, gen. 12....	..	..	..	..	..	..	2	..	1	..	..	3	12	3.17
♀ 5513, $+4\frac{1}{4}$, and ♀ 6955, +4, gen. 12.....	..	..	..	..	..	..	..	1	1	..	..	2	5	3.37
Totals.....	1	..	4	2	2	9	14	11	12	16	2	73	219	3.17

children of ♀5513, produced by breeding her children brother with sister, those children all having been sired by the same wild rat. Her grandchildren include 41 hooded and 107 non-hooded young. The hooded young range in grade from $+1\frac{1}{2}$ to $+4$, their mean grade being $+3.05$, a considerable regression from the grade of the grandmother, which was 4.25.

Hooded rats of the same grade and generation as the grandmother, when bred with each other, produced young of mean grade $+3.84$. (See table 10, Castle and Phillips.[2]) The mean of the extracted hooded grandchildren in this case (being 3.05) shows a regression of 0.79 from that expected for the uncrossed hooded race. From the extracted hooded grandchildren of ♀5513, produced as just described by a cross with a wild male, 7 individuals, 2 males and 5 females, were selected for a second cross with the wild race. They ranged in grade

[2] Comparison should have been made with generation 11 offspring, whose mean was 3.91 not 3.84. This would make the regression 0.86, instead of 0.79.

TABLE 142

Table 142 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats (table 141) with wild rats. The hooded grandparents were themselves grandchildren of ♀5513, + 4¼, generation 10, on the side of both parents.

Hooded grandparents.	Grade of hooded grandchildren.									Total hooded.	Total non-hooded.	Means of hooded.
	2	2¼	2½	2¾	3	3¼	3½	3¾	4			
♀ 9619, + 2	...	...	...	...	...	1	1	...	...	2	8	3.37
♂ 9686, + 2¾	...	...	...	...	1	1	2	1	...	5	28	3.40
♀ 9620, + 2¾	1	1	2	1	1	2	2	3	...	13	24	3.06
♀ 9729, + 2¾	...	...	...	...	...	1	4	4	1	10	22	3.62
♂ 9727, + 3	...	1	...	2	3	2	11	7	4	30	104	3.47
♀ 9728, + 3	...	...	1	...	1	5	6	8	1	22	68	3.55
♀ 9621, + 3¼	...	...	...	1	...	1	2	7	5	16	42	3.70
Totals	1	2	3	4	6	13	28	30	11	98	296	3.47

from + 2 to + 3¼. (See table 142.) They produced several litters of young of the same character as the first F_1 young, all being similar to wild rats in appearance, except for the frequent occurrence of a white spot on the belly. These second F_1 young were at weaning time mated, brother with sister, in breeding-pens, precisely as had been done with the first F_1's. They produced 394 second F_2 young, of which 98 were hooded and 296 non-hooded, a perfect 1:3 ratio. The hooded young varied in grade from + 2 to + 4, as shown in table 142, the data there being given for each family separately as well as for all combined in the totals. One family was very like another as regards the character of the hooded young, except that the higher-grade grandparents had grandchildren of slightly higher grade. Thus the average of all the 98 hooded young was + 3.47, but the average of those descended from the 3 grandparents of lowest grade was *less* than this, while the average of those descended from the 3 grandparents of highest grade was *greater*. This is just what had been observed throughout the entire selection experiments. (See Castle and Phillips.)

If we weight each of the grandparents in table 142 in proportion to the number of its hooded grandchildren, then the mean grade of all the grandparents is + 2.95. Since the mean grade of all the 41 first F_2 hooded grandchildren, from which these 7 were chosen, was + 3.05, it will be seen that these 7 are, so far as grade is concerned, fair representatives of the 41, being in fact of slightly lower mean grade. It is therefore all the more striking that their grandchildren, the second F_2 hooded young (table 142), are of higher grade. They regress in an *opposite* direction to that taken by the first F_2 hooded young. Thus the original hooded ancestor (♀5513) was of grade 4.25. The grade of hooded young expected from such animals is 3.84. What she produced

in F_2 following a cross with the wild male, was young of mean grade 3.05. Seven of these of mean grade 2.95 produced a second F_2 containing hooded young of mean grade 3.47. This is a *reversed* regression of 0.52 on the grade of their actual hooded grandparents, or of 0.42 on the group from which their grandparents were chosen. Their mean lies about midway[3] between that which would have been expected from the original hooded female (5513) had no crossing with wild rats occurred and that which was observed in the first F_2.

Obviously these facts do not harmonize with the assumption that the regression observed in the first F_2 was due to loss of modifying factors accumulated during the ten preceding generations of selection; for no further loss occurs in the second F_2. On the other hand, a partial recovery is made of what was lost in the first F_2. This suggests the idea that that loss may have been due to physiological causes non-genetic in character, such as produce increased size in racial crosses; for among guinea-pigs (as among certain plants) it has been found that F_1 has an increased size due to vigor produced by crossing and not due to heredity at all. This increased size persists *partially* in F_2, but for the most part is not in evidence beyond F_1. I would not suggest that the present case is parallel with this, but it seems quite possible that similar non-genetic agencies are concerned in the striking regression of the first F_2 and the subsequent reversed regression in the second F_2.

Whatever its correct explanation may be, the *fact* of the reversed regression in a second F_2 is very clear, as other cases than those already discussed will show.

A hooded rat of grade +4 and generation 10, ♂6348, had by a wild female several young of the character already described for the young of ♀5513. These, mated brother with sister, produced a first F_2 (table 141) of 90 rats, 22 of which were hooded, 68 being non-hooded, again a good 1:3 ratio. The hooded young ranged from +2 to +4 in grade, their mean being 3.28. Of the 22 hooded individuals, 1 male and 7 females were mated with wild rats to obtain a second F_1, and the second F_1 animals were then mated brother with sister to obtain the desired second F_2. The character of this is shown family by family in table 143. It contained 497 individuals, of which 121 were hooded and 376 non-hooded, a ratio of 1:3.1. The weighted mean of the 8 selected grandparents is 2.93, which is 0.35 below the mean of the 22 first F_2 hooded animals which they represent. The mean of the second F_2 hooded young is 3.22, which indicates a *reversed* regression of 0.29

[3] In *The Scientific Monthly* (Jan. 1916) I have stated that a second cross showed "a return to about what the selected race would have been had no crossing at all occurred." This is obviously inaccurate and should be corrected. It rests on a comparison with the combined average of both the older and the more recent experiments. [MacDowell devotes half a page to demolishing the statement already corrected here.]

on the grade of the grandparents, but shows no significant difference from the mean of the grandparental group (3.28).

TABLE 143

Table 143 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats (table 141) with wild rats. The hooded grandparents were themselves grandchildren of ♂6348, + 4, generation 10, on the side of both parents.

Hooded grandparents.	Grade of hooded grandchildren.										Total hooded.	Total non-hooded.	Means of hooded
	1¾	2	2¼	2½	2¾	3	3¼	3½	3¾	4			
♂ 9639, + 2.....	1	2	1	...	3	6	4	15	6	1	39	110	3.24
♀ 9704, + 2¾.....	...	1	...	...	...	1	...	4	...	...	6	16	3.17
♀ 9765, + 3.....	...	...	...	...	...	...	...	1	...	...	1	10	3.50
♀ 9747, + 3¼.....	1	7	...	1	1	7	1	4	4	1	27	76	2.90
♀ 9703, + 3½.....	...	...	1	1	2	2	1	5	2	2	16	47	3.28
♀ 9705, + 3½.....	...	...	...	...	1	5	1	4	8	2	21	74	3.48
♀ 9748, + 3½.....	...	...	...	...	1	2	1	2	3	...	9	40	3.36
♀ 9796, + 4.....	...	...	...	...	...	...	...	...	1	1	2	3	3.87
Totals.....	2	10	2	2	8	23	8	35	24	7	121	376	3.22

All except one of the 8 families classified in table 143 show unmistakably the reversed regression. This exceptional family consists of the grandchildren of ♀9747. They have a mean grade of 2.90, substantially the same as that of the entire group of grandparents, but considerably lower than that of their own hooded grandmother. Apparently she did not come up genetically to her phenotypic grade. This the other grandparents of the group did. For those of lowest grade (2, 2¾) produced lower-grade hooded grandchildren than did the grandparents of highest grade (3½, 4), as was found to be the case also in table 142.

We may next trace the inheritance of the hooded character through a third but smaller family produced by two successive crosses with wild rats, the hooded character in this case being derived from ♀6995, grade + 4, generation 12. The character of her first F_2 descendants is shown in table 141. They consist of 5 hooded and 27 non-hooded individuals. The mean grade of the hooded young is 3.51, but the number of these young is too small to make this mean of much significance. One of the hooded young (♂9660, + 3¾) was mated with a wild female to secure a second F_1 generation and from this in due course was produced the second F_2 generation (table 144). It consisted of 21 hooded and 44 non-hooded young. The hooded young showed the usual range (2 to 4). Their mean grade was 3.50, substantially identical with that of the first F_2 animals, but 0.25 below that of the actual hooded grandparent. This family history is less satisfactory than the two already discussed because of the smaller numbers which it includes. It

contains nothing contradictory to the interpretation already given, though reversed regression is not in this case in evidence.

TABLE 144

Table 144 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats with wild rats. The hooded grandparent, ♂ 9660, + 3¾, was a grandson of ♀ 6955, + 4, generation 12, on the side of both parents. The hooded grandparent ♂ 9711, + 3½, was a grandson, on the side of one parent, of ♀ 5513, + 4¼, generation 10, and on the side of the other parent, of ♀ 6955, + 4, generation 12. (See table 141.)

Hooded grandparents.	Grade of hooded grandchildren.									Total hooded.	Total non-hooded.	Means of hooded.
	2	2¼	2½	2¾	3	3¼	3½	3¾	4			
♂ 9660, + 3¾.....	1	...	...	1	1	2	5	9	2	21	44	3.50
♂ 9711, + 3¼.....	...	...	1	2	2	4	4	2	1	16	33	3.28
Totals.....	1	...	1	3	3	6	9	11	3	37	77	3.40

In two cases F_1 females could not be mated with brothers and so mates were taken from other families. Thus "mixed F_1 matings" were made between children of 5513 and 6600 and children of 5513 and 6955. (See table 141.) The former mating produced 3 hooded and 12 non-hooded "first" F_2 young; the latter produced 2 hooded and 5 non-hooded "first" F_2 young. The grade of the hooded young produced by these mixed matings was not different from that of brother-sister matings, so far as the small numbers permit one to judge. One of these mixed matings was carried into a second F_2 generation. The first F_2 hooded ♂ 9711, + 3¼, was mated with a wild female, and the young were bred, brother with sister, producing 16 hooded and 33 non-hooded young. (See table 144.) The mean grade of the 16 hooded young was 3.28, substantially the same as that of the first F_2 hooded grandparent. No additional regression through loss of modifiers (or other agency) is here in evidence. The result is the same as that observed in families wholly unmixed. The attention of my pure-line critics, who think that in our mass selection experiments insufficient attention has been given to individual pedigrees, is particularly directed to the foregoing case.

Having now discussed each family history separately, we may combine all the second F_2 families in one table, in order to get a clearer impression of the results as a whole. (See table 145.) The second F_2 generation thus combined includes 256 hooded and 749 non-hooded individuals, a ratio of 1:2.9, an unmistakable mono-hybrid Mendelian ratio. The mean grade of the hooded individuals is 3.34. The weighted mean grade of their hooded grandparents was 3.02, which indicates a *reversed regression* of 0.32 for the entire second F_2 group of hooded animals.

TABLE 145

Table 145 is a combination of tables 142 to 144, in which the second F_2 young are classified according to the grade of their first F_2 hooded grandparent.

Grade of hooded grandparents.	Grade of hooded grandchildren.										Total hooded.	Total non-hooded.	Means of hooded.
	1¾	2	2¼	2½	2¾	3	3¼	3½	3¾	4			
2	1	2	1	...	3	6	5	16	6	1	41	118	3.25
2¾	...	2	1	2	1	3	4	12	8	1	34	90	3.29
3	...	...	1	1	2	4	7	18	15	5	53	182	3.48
3¼	1	7	...	2	4	9	6	10	13	7	59	151	3.22
3½	...	...	1	1	4	9	3	11	13	4	46	161	3.39
3¾	...	1	...	...	1	1	2	5	9	2	21	44	3.50
4	...	...	...	...	...	...	...	...	1	1	2	3	3.87
3.02	2	12	4	6	15	32	27	72	65	21	256	749	3.34

Classified according to the grade of the (first F_2) grandparent, they show a correlation between grade of grandparent and grade of grandchild. The lower-grade grandparent has lower-grade hooded grandchildren, and the higher-grade grandparent has higher-grade hooded grandchildren. This shows that the variation in grade is (in part at least) *genotypic*. As the experiment yields no evidence that the variation in the hooded character is due to independent modifying factors, there remains no alternative to the conclusion that the single genetic Mendelian factor concerned fluctuates in genetic value. Fluctuation accordingly is not exclusively phenotypic, as DeVries and Johannsen have thought, but may be genetic also. Hence racial changes may be effected through selection by the isolation of genetic fluctuations, as well as by the isolation of mutations. Moreover, genetic fluctuation makes possible *progressive change* in a particular direction, repeated selection attaining results which it would be quite hopeless to seek by any other means.

2. *The progeny of "mutant" crossed with wild rats.* (From Publication 241, p. 173.)

Castle and Phillips described, under the name of "mutants," 2 rats of the plus-selection series of extremely high grade. They proved to be heterozygotes between the average condition of the plus-selected race at that time, about +3.75, and a new condition, not previously known in our hooded races, but resembling that seen in "Irish" rats, which are black all over except for a white spot on the belly and would be classed on our grading scale as about +5½. In later generations we secured animals homozygous for the darker condition just described (that of Irish rats). The homozygous "mutant" race proved to be very stable in color pattern, varying only from 5¼ to 5¾, with a majority of animals graded 5½. Attempts to alter the modal condition of the race

by selection have thus far proved futile because of our inability to increase the race sufficiently to afford a basis for selection. Its inbredness and its feebleness are perhaps causally related.

The suggestion was made that the change from our plus-selected race, which had occurred in the mutant stock, might be due to some supplementary modifying factor, not to a change in the hooded factor itself.[4] If so, a cross with a race lacking the hooded factor or its "modifiers" might serve to demonstrate their distinctness by separating one from the other. A wild race seemed best suited for a test of this hypothesis, since it would be free from suspicion on the possible ground of harboring either the hooded pattern or its supposed modifier, which had converted the hooded pattern into the mutant. It was to be expected, if the hypothesis were correct, that the mutant character was hooded plus modifier; that then a cross with wild should produce in F_2 *hooded* young (lacking the modifier) as well as mutants and selfs. But if the mutant race had arisen through a change in the hooded factor itself, then the cross should produce only mutants and selfs, without hooded young in F_2. Crosses have now been made on a sufficient scale to show beyond question the correctness of the latter alternative, which is entirely in harmony also with the results described in the preceding parts of this paper.

Six homozygous "mutant" females of grade $+5\frac{1}{2}$ were mated with wild males of the same race described in Part I. They produced 46 young, all gray like wild rats and of grades as follows:

Grade	$5\frac{1}{2}$	$5\frac{3}{4}$	$5\frac{7}{8}$	6
No.	1	15	7	23

Exactly half of the 46 F_1 rats bore no white spot, *i. e.*, were of grade $+6$. Seven more bore only a few white hairs (grade $5\frac{7}{8}$). The remainder were very similar to the mutant parent in grade.

Several matings were made of the F_1 rats, brother with sister, which produced 212 F_2 young. About a quarter of these were black (non-agouti), the rest being gray (agouti). Both sorts included about equal numbers of individuals with and without white spots. No difference was observed in this respect between the progeny of spotted and of unspotted parents. Table 158 shows the F_2 young grouped family by family according to grade. Three of the four families are descended from a single mutant grandparent; the fourth family is descended from two different mutant grandparents which were bred simultane-

[4] This also is MacDowell's view. He says, p. 734: "The newly discovered factor acts independently of the other factors, is not modified by them, and does not modify them. Being the one difference between the mutant and the plus race at the time the mutant appeared, this factor affords a crucial test for the interpretation of the modifications that result from crosses."

ously to the same wild male in the same cage. The 10 F_2 young of this family may have been produced either by full brother and sister, or by half-brother and half-sister; it is uncertain which. All other F_2 young were produced by brother-sister matings.

TABLE 158

Table 158 shows the classification of the F_2 young obtained by crossing homozygous "mutant" with wild rats.

Mutant grandparents.	Grade of offspring.						Totals.
	5	5¼	5½	5¾	5⅞	6	
♀ 0630, + 5½	...	3	12	9	2	20	46
♀ 0698, + 5½	1	2	22	29	1	59	114
♀ 0694, + 5½	...	...	12	11	...	19	42
♀ 0630, + 5½, or 0636, + 5½	...	1	3	1	...	5	10
Total	1	6	49	50	3	103	212

It will be observed that the F_2 young (table 158) which are white-spotted are in no case hooded. Their range of variation does not fall beyond that of the uncrossed mutant race. It is certain, therefore, that the "mutant" condition is not *hooded plus an independent Mendelian modifier*. It is a changed form of white-spotting, alternative to the form of spotting found in the race from which it was derived (the plus-selection series, generation 10). It is, without much doubt, also alternative to the self condition of wild rats, though fluctuation in grade obscures the segregation, which may, very likely, be imperfect. This serves to confirm the general conclusion that throughout the entire series of experiments with the hooded pattern of rats we are dealing with quantitative variations in one and the same genetic factor.

Conclusion

I have now presented the evidence which has led me to reject the hypothesis formerly held tentatively that modifying factors were largely concerned in changes produced in the hooded pattern of rats under repeated selection. This evidence seems to me to admit of only one consistent interpretation, that a single variable genetic factor was concerned in the original hooded race, that a changed condition of this same factor was produced in the minus race, and another changed condition in the plus race, and a third appeared in the mutant race. All are allelomorphs of each other, and of the non-hooded or self condition found in wild rats, yet all tend to modify each other in crosses. The character has a high degree of genetic stability, yet is sub-

ject to continuous genetic fluctuation. I have been unable to produce or to discover any race of spotted animals which is free from genetic fluctuation, though I have made an extended search. If MacDowell or any one else has discovered such a race, let it be produced.

It does not, of course, follow that because white spotting in rats is capable of indefinite modification through selection, therefore all heritable characters are equally capable of modification. Physiological limitations no doubt often limit the modifiability of characters. A sugar-beet can not be produced which is *all* sugar or much over 25 per cent. sugar. There has to be retained a plant mechanism for the production of sugar, a beet. Neither is it to be expected that the thorax of *Drosophila* can be decorated with an indefinite number of extra bristles. The bristles have to be attached to something, and the thorax of *Drosophila* is finite in size. It is not necessary to suppose that hypothetical modifying factors have been used up simply because variation ceases to progress in a particular direction. For no one, I suppose, would contend that variation is equally easy in all directions and in all characters. De Vries has taught us the significance of one-sided variation and we have become familiar with recurrent types of variation which are encountered first in one species and then in another. Such cases show that different kinds of germplasm are similar in structure and likely to undergo similar changes. But what happens to these spontaneous variations when once they have put in an appearance depends on external agencies, man or other factors in the struggle for existence. The modern study of evolution has indeed emphasized the importance of spontaneous internal changes in producing variations, but we still have to reckon with selection, natural and artificial, in determining the survival of variations as well as in controlling their magnitude and the direction of their further variation.

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FIELD TESTS OF THEORIES CONCERNING DISTRIBUTIONAL CONTROL[1]

THE conditions of animal distribution and the causes of these conditions are facts which concern intimately the problems of the persistence and of the evolution of species. The present writer believes that the field naturalist is in a position to contribute in large measure toward the solution of these problems, and it is the purpose of this paper to show how comparative studies in the distribution of species may throw light not only upon the nature of the environmental complex, but also on the relative importance of its various component factors.

Some simple facts of distribution which are of common observation, and which were early recorded by the systematic zoologist, are: (1) that each animal occupies a definite area, that is, has a habitat or range, which is distinctive enough to be included among the characters of the species and described along with its habits and the features of its bodily structure; (2) that some species (and even some of the higher systematic groups) range widely, and cover great extents of country, while others are extremely local or restricted in their distribution; and (3) that, notwithstanding considerable variation in this degree of distributional restriction, many species (or higher groups) are found nearly or entirely to coincide in range, so that sets of species, of varying ranks, may be recognized distributionally, as constituting realms, zones, faunas, subfaunas, associations, etc.

Perhaps the most prominent delimiting factor, and the one which has been emphasized through repetition in the early systematic writings, is the obvious one of physical barriers—represented by bodies of water in the case of the terrestrial species and by land in that of the aquatic. The majority of animals inhabiting islands and seas are specialized in such a manner as to be hemmed in by the limits of their respective habitats. Individuals overstepping the barrier in either case are subject to prompt destruction. This obvious type of distributional control has always been and will remain an important one for consideration; but with the acquisition of detailed knowledge regarding the distribution of animals on large continental areas, naturalists have been led to propose many other factors which

[1] Contribution from the Museum of Vertebrate Zoology of the University of California.

have seemed to them to prevent the random and unrestricted spreading of animals over the surface of the land. The following is a list of the factors which various writers have nominated as affecting the distribution of the higher vertebrate animals. This list is complete only to the extent that my own examination of the literature is so. Many of the items have been found in dissertations upon bird migration, which is, of course, but one phase of the general subject of distribution.

Vegetation.
Food supply, kind and quantity.
Rainfall.
Humidity of the air (relative or absolute).
Wetness or dryness of the soil.
Barometric pressure, or altitude.
Atmospheric density.
Safety of breeding places.
Availability of temporary refuges.
Water (to land species).
Land (to aquatic species).
Nature and availability of cover, or shelter from enemies.
Nature of the ground (coarse or fine soil, or rock).
Insolation, or light intensity.
Cloudiness.
Temperature: in general; mean annual; of winter; of period of reproduction; of hottest part of year.
Interspecific pressure, or competition, or race antagonism.
Parasitism.
Individual, or racial, preferences.

It is at once plain that some of the items enumerated are extremely complex, and that the most superficial analysis will show some duplication among them. For example, the factor of vegetation as influencing the distribution of different mammals resolves itself principally into the elements of food-supply and shelter, and, subordinately in most cases, into those of temperature, humidity, and nature of the soil. As some of the suggested factors may really never function in any vital degree as supposed, the total number of really critical factors is probably smaller than the total of the items just listed. Time could not here be taken to discuss the intrinsic nature of each elemental factor, even if the writer were equipped to handle such a variety

of subjects; for such a discussion would in most instances lead directly into physics and chemistry, and into a study of the physiological processes of the animals affected by each of these factors. I should, however, like to dispose at once of one of the "factors" listed, and which I hear and see repeatedly cited as a cause of restriction in distribution—particularly in that of birds.

Many people claim to see in the facts of distribution only the operation of a *preference* on the part of each animal—by virtue of which, if a heterogeneous lot of animals were introduced into an area presenting diverse conditions, each species would *choose* its "natural" surroundings and rapidly allocate itself in a normal way. I grant that such a choice would almost certainly be made. In fact the hypothesis is being proved continually all over the country in connection with the migration of birds. Scores of species travel north in the spring to countries for a preceding interval unoccupied; and while, roughly speaking, they travel together, and arrive together, they segregate themselves, immediately on their arrival, and repair to separate sorts of ground, each species by itself: the pipits to the prairie, the water-thrushes to the streamside thicket, the black-poll warblers to the spruce forest, and so on. We have here an obvious choice exercised in the selection of habitats. But does this segregation of species by exercise of "individual preference" in a uniform direction change the nature of the problem in any fundamental way? Should we not here recognize merely a character in the cerebral equipment of each race, which, like every external peculiarity in its structure, is in considerable measure the result of protracted impress upon the organism from the environmental complex of factors to which the race has been subject through past time? There is no other additional factor than those environmental ones (plus the intrinsic fixedness of the species, within certain limits of plasticity, and the "evolutionary momentum") to be called into account.

As to the mechanism of geographic limitation, the adjustments to the various critical factors are inevitably forever in process, though reduced to a minimum at times of slow environmental change. The refined method of individual "preference" or "choice" is superior to the wasteful process of wholesale destruction which would be experienced by individuals finding themselves out of place as the result of a haphazard selection of

locality. The frontier individuals, those on the margin of the habitat of the species, may not prosper as greatly, or reproduce as prolifically, as those in the metropolis of their species; but they certainly do not, as a rule, beat themselves to death individually against their limiting barrier, of whatever nature it may be.

To resume the main topic of this discussion, I shall attempt to show that it is possible from field observation to indicate in the case of certain species, some, at least, of the factors which control their distribution; and further that we who live in California have splendid opportunities to gather and examine data by means of which the general laws of animal distribution can be determined. An area within comparatively easy reach presents a wide diversity in topographic and climatic features. Occupying this area is an abundant complement of the higher vertebrate classes. Within the political limits of the state, systematists now recognize the presence of 388 species of mammals, 543 of birds, 79 of reptiles and 37 of amphibians. We have plenty of material to work with. I shall proceed to discuss a few selected species about which we seem to have knowledge enough to warrant provisional inferences.

THE CASE OF THE OREGON JAY

The Oregon jay (*Perisoreus obscurus*), a close relative of the Canada jay, or whisky-jack, occurs in California only in the northern third of the state. Even there it is very local in its occurrence and absolutely non-migratory. On the Warner Mountains, Modoc County, it ranges from the highest parts down to 7,000 feet altitude. On Mount Shasta it ranges from near timberline down to about 6,000 feet altitude. It is absent for a long distance to the west, through the Trinity mountain mass, but it recurs along the seacoast of Humboldt County, within fifteen miles of the ocean. And here is the curious point: along this coast strip it does not range higher than 300 or 400 feet above sea level, although there are mountains not far inland which rise to an altitude of several thousand feet. Let us look into this case for the purpose of determining the factors responsible for this interrupted range.

The Oregon jay, like most members of the crow family, is not restricted in diet. It eats a great variety of both vegetable and animal substances; its food varies in character according to season and local conditions. The supply of any particular kind

of food is not likely, therefore, to be a controlling factor in its distribution.

The bird is a forest dweller. Its equipment as regards manner of flight and course to take in case of attack by enemies is adjusted to a forest habitat, and nowhere within the writer's knowledge does this jay extend its range beyond the limits of woods of some sort. Although somewhat predaceous itself, it has regular enemies among hawks and owls, for protection from which it makes use of forest vegetation. This factor of forest cover, then, must be counted as essential. But the range of the bird is not continuous wherever forests extend.

In the interior of California it does not descend below a certain altitude. Now three other factors in its distribution are quite obviously connected with that of altitude, namely, barometric pressure, atmospheric density, and temperature. But when we take into account the fact that the Oregon jay exists at or close to sea level around Humboldt Bay, the first two factors, those of pressure, and air density, are instantly eliminated, because of the obvious fact that the bird successfully maintains itself in localities of widely differing altitude where these factors are thus extremely diverse.

With reference to temperature, we know without recourse to instrumentation that there is a decrease upwards at an average rate of 3 to 4 degrees F. per thousand feet. If, then, the bird is limited downwards at a critical point, the inference apparently follows that temperature is the determining factor, and this conclusion is inevitable if we consider only Mount Shasta and the Warner Mountains. But the bird's occurrence at Humboldt Bay complicates the problem. In order to reconcile these facts of distribution we must look into the situation with reference to season. On doing so we discover that the home of the Shasta and Warner jays is subject to severe winters with heavy snow, very much colder than the winters at Humboldt Bay, where the climate is equable and snow rarely falls. But the summer temperature at Humboldt Bay is well known to be much cooler than that of even somewhat higher regions in the interior, up to an altitude of at least 4,000 or 5,000 feet, because of the eastward moving air-currents, which are coolest where they first leave the sea surface and warm up as they pass farther and farther inland. We are therefore led directly to the final inference that the summer temperature at sea level about Humboldt Bay

closely approximates the summer temperature at from 6,000 to 9,000 feet on Mount Shasta and above 7,000 feet on the Warner Mountains. In these three areas, the air is cooler in summer than in the interlying areas and thus better adapted to the finely adjusted requirements of the Oregon jay. *Summer* temperature, between certain degrees, is one critical factor.

Three more factors present themselves for consideration in connection with the Oregon jay, those of humidity, rainfall and cloudiness. Humboldt Bay lies in the most humid and continuously rainy section of California. Mount Shasta and the Warner Mountains are relatively arid, the latter most notably so. It would appear, therefore, that humidity, rainfall and cloudiness had little or nothing to do with cutting off the range of this bird, though one or other of these factors may have been responsible for the very slightly darker tone of color which distinguishes the coast jays (subspecies *Perisoreus obscurus obscurus*) from those in the interior (*P. o. griseus*). But, however this may be, it is clear that temperature must dominate greatly over the three factors named in checking dissemination.

In summary, we may therefore dispose of the following factors as having little or no effect on the distribution of the Oregon jay as a species: the nature or quantity of its food supply, atmospheric density and pressure, cloudiness, rainfall, humidity of the air or soil, and winter temperature. This eliminates all but the two factors: shelter of a sort provided by the forest habitat, and temperature of the summer season.

THE CASE OF THE CONY

The cony or pika is a mammal represented in California by four quite similar races (*Ochotona taylori, O. schisticeps schisticeps, O. s. muiri,* and *O. s. albatus*), which agree distributionally in occupying a very restricted habitat along high mountain crests. I know of no place in central California where conies range below an altitude of about 8,000 feet, and they range upwards to fully 12,000 feet in the vicinity of Mount Lyell. They thus occupy an altitudinal belt between extremes 4,000 feet apart. With regard to zones of vegetation conies live from considerably below timberline to considerably above timberline. Extended observation shows that their existence is in no way correlated with that of trees or shrubs of any sort. Like their relatives, the rabbits, they feed entirely on low vegetation, biennials mostly;

but unlike most kinds of rabbits they are strictly dependent for safety from enemies upon rocks, especially where these are loosely piled as in talus slopes and so afford deep retreats within their interstices. The whole equipment of a rabbit is clearly adapted to foraging in the open, its keen hearing and eyesight quickly warning it of the approach of enemies, and giving it time to escape by means of its unusual running powers. But the cony is equipped in a very different way, as it has relatively small ears and eyes, and small hind legs. It is compelled to forage close to or beneath cover. In fact in field observations it is rarely seen on the move except momentarily, and then only between or beneath angular granite blocks, where it grazes on such little patches of vegetation as are within immediate reach.

It is clear from numerous observations that the cony is sharply restricted in a large part of its range by the rock-pile habitat. Even at favorable altitudes it is not found away from this refuge. There are obviously, however, one or more additional factors in its distribution. In many parts of the Sierras, talus slopes occur from near the highest summits down to the foothills. As examples of these, one may cite the vast earthquake taluses of the Yosemite Valley proper, which occur almost continuously down to and below the 4,000-foot contour. These taluses have been searched diligently both by trapping and hunting, without our naturalists finding a trace of conies below 8,000 feet. The animals are easy to detect, by reason of their characteristic cry, uttered at any time during the day, though more particularly in the morning and the evening, and by the accumulations of their feces, the pellets constituting which are, in size, shape and texture, unlike those of any other mammal. What is it, then, that limits the conies downward on the western flank of the Sierras, where their necessary rock habitat is continuous, and where food of the right sort is also continuous? Let us try barometric pressure, and atmospheric density, which may properly be considered together. These conditions change sensibly with altitude and, if we take into account California alone, the facts would seem to entitle them to serious consideration as active delimitors of the conies downward. But as we trace the range of the conies far to the northward we are led to a different conclusion. The altitudinal limits of their range is found to descend quite regularly towards the north, until, in the case of one race, even sea level is reached, at Bering Sea. Clearly, conies, generically, are

thus proven not to be affected by atmospheric pressure, or by atmospheric density, at least in as far as it is modified by altitudes up to 12,000 feet. The same fact—depression of range towards the north—discloses a third concomitant of altitude, which is also a concomitant of latitude, namely, temperature, and this is beyond doubt the determining factor. As the isotherms dip toward sea level to the northward so does the range of the genus *Ochotona*. We have, therefore, by study of geographical distribution in this case established two important controlling factors, namely (1) safety refuges of a sort provided by talus slopes and glacial moraines; (2) temperature, at least downward below the degree, correlated in the mountains of California by a mean annual or summer computation or for a briefer period at the time of reproduction, with an altitude of eight to twelve thousand feet, according to latitude, slope exposure and air currents.

It is not possible for one to say from the data in hand what the direct controlling factors of the upward limits of the cony's range may be. Taluses extend up to the highest peaks, but there is no growth of grass above about the 12,000-foot contour even on the most favorable slopes. As the disappearance of the cony in the higher altitudes is coincident with the disappearance of its food, it appears as if failure of food alone were the delimitor here; but we have no way of showing that even if food did continue the cony would be restricted upward, as it certainly is downward, by a change in temperature beyond some critical point. The cause of its delimitation downward, however, remains clear.

THE CASE OF THE ROSY FINCH

In the case of the bird called generically *Leucosticte*, or rosy finch, we find a condition astonishingly similar to that of the cony. In fact almost the entire preceding account could be made relevant here, by merely substituting the term rosy finch for cony. The ranges, altitudinal and geographical, of the two animals are almost identical. The only obvious differences appear in their ecologic relations, and consist in the lesser dependence of the bird upon shelter and in the dissimilar nature of its food. The rosy finch forages gregariously on the open slopes, near timberline and above, though its nest is hidden away in the clefts of rock ledges and taluses. It shuns the trees

and bushes even where it ranges well below timberline. It feeds winter and summer upon seeds of dwarfed vegetation, including those of grass and herbs of various sorts. As far as I can see, its food and feeding habits are identical with those of such other fringillids as goldfinches and siskins. Yet the leucosticte, by the same tests as were used with the cony, is beyond any contention limited downward by an increase of temperature. We find the bird to possess various adaptive features in common with certain arctic finches, such as tufts of bristle-like feathers over the nostrils to prevent fine snow from entering. These enable the bird to spend the long winter on the cold wind-swept ridges, but at the same time would hardly prevent the bird's dropping to warmer climes if the heat were not a strongly deterrent factor.

Cases of coincidence, as instanced by that of the cony and leucosticte, among animals of widely different powers of locomotion and ecologic position, are the rule, not the exception, and impel the observer to belief in the efficacy of the controlling factor above mentioned.

The Case of the Redwood Chipmunk

The redwood chipmunk (*Eutamias townsendi ochrogenys*) is an animal confined to a very narrow but exceedingly long distributional area extending south from the Oregon line as far as Freestone, Sonoma County. Throughout this belt it is conspicuously numerous, and is usually the only species of chipmunk present, so that the limits of its range have been easy to mark definitely along the several lines explored. This rodent, by various geographic tests similar to those I have recounted for other birds and mammals, is clearly delimited away from the coast at the bounds of the well-known fog-belt to which the redwood tree and numerous other plants as well as animals belong. The chipmunk, however, depends in no way upon the redwood or any other one plant species as far as I can see, but feeds upon a great variety of seeds and fruits, like many of its congeners elsewhere.

That temperature is also a delimiting factor is shown in parts of the range of the redwood chipmunk. But atmospheric humidity or cloudiness or rainfall, factors which I have in this case failed to dissociate, together constitute or include the chief controls.

THE CASE OF THE BELTED KINGFISHER

It is to be observed that specialization for getting a particular kind of food invariably brings with it restriction of range to the territory providing that kind of food. The northwestern belted kingfisher (*Ceryle alcyon caurina*) is a good example of this. In California we find this bird present at various times of the year both along the seacoast and along various fish-supporting streams, from the Colorado River to the Klamath River and up the mountain streams to at least as high an altitude as Yosemite Valley. The kingfisher is seen during migration in many places away from streams, but it tarries at such times only where its natural diet can be procured, as, on occasion, at fish ponds in city parks. There is a unique instance of a kingfisher observed on the desert catching lizards, but exceptional occurrences of this kind are of course not to be given consideration in making generalizations.

It is observable further in regard to this species of kingfisher, that it must have earth banks in which to excavate its breeding tunnels. Lack of these along any stream, otherwise favorable, prevents the bird from staying there through the season of reproduction. Furthermore, there is also obvious temperature restriction; for, given a fish-producing stream, with banks apparently well suited for excavation of nesting places, such as is the Colorado River and its distributaries, and the summer temperature must be at least below that of southern California south of the 35th parallel. That all such streams are well supplied with kingfishers in winter, and are forsaken only during the hot summer, seems to show that a relatively cool temperature is for them in some way or another essential to successful reproduction.

We find, then, in the case of the belted kingfisher, that the factors of a requisite kind of food, and a requisite kind of nesting place, both having to do with the structural powers and limitations of the bird, together with the factor of the temperature of the summer season, are those that account for the distribution of the species within the state of California, as we find it.

THE CASE OF THE MEADOWLARK

The western meadowlark (*Sturnella neglecta*) is a bird of relatively omnivorous diet. Note that I say relatively, for the word omnivorous unmodified would apply only to such an ani-

mal as would eat the sort of food that any animal eats, and this is an obvious impossibility for the meadowlark when we consider such uncommon articles of diet as wood and petroleum. Compared with many other birds, the meadowlark does use as food a very wide range of plant and animal objects. This food, however, is restricted to a particular habitat source, namely to the meadow. The bird's entire equipment specializes it for successful food-getting and for escape from enemies upon a grassy plain or meadow. And it is a matter of common observation that its range is sharply delimited in most directions at the margin of the meadow habitat, as where this is interrupted by forest, brushland, marsh, rock surface or sand flat. This is a conspicuous example of what we may call associational restriction. But it is not the only way in which the meadowlark is hemmed in. In this connection California again provides critical distributional evidence.

We find meadowlarks occupying practically every appropriate meadow, large and small, from the Mexican line to the Oregon line and from the shores of the Pacific to the Nevada line, *except* above a certain level on the higher mountains. In traveling up the west flank of the Sierras, and this I have now verified along three sections, meadowlarks cease to be observed at approximately the 4,500-foot level, and this in spite of the fact that above that altitude meadows are found which are to all appearances ideal for meadowlark requirements. I need only refer to such seemingly perfect summer habitats as Monache Meadows and Tuolumne Meadows. And though, in the winter these would be uninhabitable, so are other meadows (as those in the Modoc region, for instance), which are in summer warm and at that season abundantly inhabited by meadowlarks. By the elimination then upon proper grounds of various factors from the list, we have left only three possible factors in this upward delimitation, namely, decreased atmospheric pressure, decreased air density and decreased temperature of the summer season. Since meadowlarks exist at corresponding altitudes in the warmer though elevated Great Basin region, and since it has been possible to eliminate positively and in a similar way the first two factors in the cases of many other birds and mammals, these factors are presumably without influence on the meadowlark; and there is left but one—temperature.

Within the state of California, meadowlarks, without the

slightest detectable subspecific modification, thrive under both the cloudy, humid conditions of the northwest coast belt and under the relatively cloudless, arid conditions of Owens Valley. Factors of humidity, of air and soil, cloudiness, and light intensity, seem to avail nothing in checking their spread. With such a degree of associational specialization as is exhibited by these birds there is little chance of a serious competitive struggle with other vertebrates, and no evidence of such has been observed. As far as California is concerned, the meadowlark's range is thus only limited associationally and zonally, that is by the extent of its particular meadow habitat and by diminished summer temperature below some critical point.

The meadowlark well illustrates some further facts with regard to distribution. In California it is unquestionably on the increase as regards total population. This is due chiefly to the great extension of habitable territory resulting from man's occupancy and cultivation of the land, bare plains, brushlands and even woods being replaced by irrigated alfalfa and grain fields. These the meadowlarks find suitable and invade because of their expansive reproductivity, and soon populate to the fullest extent permitted by the minimum annual food supply. In other words, associational barriers have moved, to the advantage of this particular bird, though at the same time to the disadvantage of endemic species of different predilections. I should estimate that the total meadowlark population in the San Joaquin-Sacramento basin is now fully three times what it was thirty years ago.

Animal distribution is not fixed. It changes with the shifting of the various sorts of barriers, and doubtless also as a result of a gradual acquisition by the animals themselves of the power to overstep barriers, as by becoming inured to greater or lesser degree of temperature. The power of such accommodation, or inherent plasticity, evidently varies greatly among different animals; and at best its operation is very slow. Many species have proved stubborn and have been exterminated, as the factor-lines, or barriers, shifted. By the shifting of, say, two critical factor-lines towards one another, the existence of a species may have been cut off as by a pair of shears.

Summary

In this paper I have enumerated various factors thought to be concerned with the control of the distribution of vertebrate animals. A number of birds and mammals have been cited to show how we may use our more or less detailed knowledge of their ranges so as to demonstrate the operation of one or several out of the many possible factors as limiters to distribution. The method employed is one of examination, comparison and elimination, applied to all parts of the margin of animals' ranges. The range of any one animal must be examined at all points of its periphery in order that all of the factors concerned may be detected. One factor may constitute the barrier in one section of the periphery of the range of a species, a totally different factor in another section.

The results of the geometric ratio of reproduction would bring about areas of occupancy in the shape of perfect circles. But we never find such symmetrical ranges. The very fact that the outlines of the ranges of animals are extremely irregular is significant of the critical nature or inexorableness of the factors which delimit them. These factors have to do with the evolution, persistence and extermination of species.

Note that we always have to take into account, in attempting to discern factors of limitation, the animal's own inherent structural equipment. This prescribes restriction at once in certain regards. Referring again to our list of suggested factors, we find the long-emphasized ones of land to aquatic species and bodies of water to terrestrial species really presenting an extreme manifestation of associational restriction. Food source, methods of food-getting and safety refuges are involved.

It is to be noted further that the factors are various and that the most important factor for one species may prove of little effect with another species. Species do not react uniformly to the same environment. It is undoubtedly always a combination of factors which accounts for an animal's geographic range in all parts of the periphery of that range. It is most certainly never one factor alone. No one will claim that temperature is the *only* delimiting agent in controlling vertebrate distribution; nor could this claim be made for humidity alone, or for food supply alone, or for safety of breeding-places alone.

Given a large continuous area, however, as upon the North

American continent, one single factor does happen to loom up as being the most frequent delimiter of distribution, or even the ultimately effective one, in greater or less degree, even though other factors be effective also. This factor is temperature. The cases cited illustrate the tenet that in some direction or another, temperature beyond certain limits, up or down, cuts off further dissemination. This is part of the basis of the life-zone idea. But, as I have tried to bring out above, this fact is in no way antagonistic to the claim that other factors, as of humidity, food supply, and shelter, also figure critically, giving a basis for recognizing faunal areas and associations. Finally, if our discussion of the subject has been sound, it is evident that data secured through field observation can be so employed as to bring results essentially similar to, and as conclusive as, those secured through laboratory experimentation.

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THE BEARING OF SOME GENERAL BIOLOGICAL FACTS ON BUD-VARIATION[1]

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I TAKE it no one denies that in the Angiosperms variations may be produced in connection with reproduction by means of buds and that these variations may be perpetuated by the same method. Practically, as horticulturists and plant breeders, we care little about the occurrence of bud-variations elsewhere in the organic world. Nevertheless, it may help in the orientation of our ideas if we remember that budding is not a rare or unconventional method of reproduction. In a generalized form, the earliest method, it has persisted throughout the plant kingdom from the most primitive to the highest and most specialized types. Sexual reproduction has not replaced it, but has been added to it. Even in the animal kingdom, though eliminated among the higher forms, it still exists as an occasional alternate method in three fourths of the phyla. Such being the case, it would seem logically to follow that variation must have been within its possibilities.

The cause, the frequency, the type, the constancy, the mechanism, of these variations are more debatable, however, and on these questions many biological facts which superficially seem unconnected, have a direct bearing. In

[1] Read before the meeting of the Society for Horticultural Science, December 28, 1916.

fact, on certain phases circumstantial evidence is the only evidence at hand.

The exact nature of the cause or causes of bud-variation can hardly be discussed profitably. We may imagine irregularities of cell division directed by combinations of unknown factors, but to describe these factors in concrete terms is at present impossible. At the same time, cause can not be neglected entirely even at present, for cause in a generalized sense is intimately connected with frequency in that vigorous perennial the question of the inheritance of acquired characters. The data on this subject are so voluminous that each for himself must give them careful conscientious consideration. Here no more can be done than to point out some of the conclusions to which I, personally, have been driven, and their connection with the subject in hand. These conclusions are:

1. Broad and varied circumstantial evidence indicates unmistakably that the inheritance of acquired characters has played an extremely important rôle in evolution.

2. Numerous experimental investigations designed to test the possibility of such inheritance directly have either failed utterly or have been open to serious destructive criticism. Direct proof of the inheritance of acquired characters is therefore lacking.

3. If conclusions 1 and 2 are to be harmonized, either modifications are fully inherited so rarely that proof that they do not belong to the general category of chance changes in constitution of the germ-plasm is impossible, or the imprint of the environment is so weak that extremely long periods of time—perhaps geological epochs—are necessary for its manifestation.

Diametrically opposed views on the inheritance of acquired characters are held tenaciously and unequivocally by equally eminent biologists. Those who concur with the Lamarckian position are nearly always the students of evolution who approach the subject from the historical or the philosophical side and who rely almost entirely on circumstantial evidence; those who adhere to the side

of Weismann are usually experimentalists whose evidence is indeed direct, but often questionable, usually capable of various interpretations, and always fragmentary. I have been bold enough to grasp both horns of the dilemma, and to plead that each is right from his point of view. My confession of faith is, the environment has been an immense factor in organic evolution, but its effects are shown either so infrequently or after the elapse of so great a time, that for the practical purposes of plant breeding we can neglect it as we would neglect an infinitesimal in a calculation. As Bergson, I think, said:

> We have been trying to prove that the hour hand moves, in a second of time.

A few words will make clear the general arguments in favor of this position, although adequate support to the thesis would require considerable time.

In the first place, it seems to me the possibility of the inheritance of acquirements must be admitted. Weismann's general contention that the chromatin of the germ-cells is the actual hereditary substance, and that the germ-cells themselves may be regarded as one-celled organisms reproducing by fission and conjugating at certain times, while the body must be considered simply an appendage thrown off from and independent of the germ-cells, is not supported merely by the embryological researches of Boveri, Kahle and Hegner on two or three animal forms, or by the ingenious ovarian transplantations made by Castle and Phillips on guinea pigs, but by all of the recent pedigree culture and cytological genetic work, botanical as well as zoological. Nevertheless it has not been and logically can not be proven that there is no way for environmental forces to produce germ-plasmic changes. Memory is just as strange a phenomenon and Semon has done biology a service by pointing out the analogy between the mechanical requirements for memory and for the inheritance of somatic modifications.

This possibility being admitted, one may well concede the plausibility of the arguments of the numerous pale-

ontologists, taxonomists and ecologists in favor of Lamarckian principles, in spite of the fact that their evidence is circumstantial. They take a comprehensive view of the actual conditions that exist among organisms, which is impossible to the experimentalist. It will not do simply to say that the manifest convergence of analogous organs in all parts of the organic world, or the wonderful adaptations of the social insects *may be explained in some other way*. Of course there may be other explanations for these phenomena; but until more satisfactory explanations are forthcoming it is rightfully a custom in science that the adequate interpretation at hand should be accepted.

On the other hand it is equally wrong for the ardent devotees of Lamarckism to clutch at every isolated case, every inadequate and abortive experiment, when judicial consideration shows not a single *unassailable* instance of the inheritance of a somatic modification. Many of these experiments have a direct bearing on bud-variation, and I shall attempt to show where they lead us.

1. *Inheritance of Mutilations.*—The most radical Lamarckians of the present day only go so far as to suppose that mutilations are inherited on very rare occasions—and they are always zoologists. Ethnology has furnished us with so many histories of mutilations of ears, of lips, of feet, of reproductive organs, long continued in the folkways of a people, that new laboratory experiments have been deserving of the ridicule they have received. Botanists have seldom had any delusions on the subject. Plants are so continually mutilated in the buffetings they receive during life, with no resulf in the next generation, that the non-inheritance of the effects of such injuries is taken as a matter of course. Yet there is occasionally one whose reason fails at the critical moment, and who holds that cuttings from the chrysanthemum with the large flower resulting from the removal of lateral branches, will produce larger flowers in the next generation than will an untreated sister plant. If not this, some equally indefensible doctrine.

2. *Effects of Changed Food Supply.*—This last example was really one of changed food supply induced by mutilation. Change of food supply by other methods has been the basis of scores of experiments, particularly on insects. Many insects are so very whimsical about what they eat that it seems possible their selective appetite may be an inherited instinct impressed by the environment of countless generations. But the total result of all experiments on them is merely to prove that a second generation may be influenced in the start they get in life by the nutrition of the mother.

The same thing is true in plants. We fertilize a pop corn to get a bumper crop of good plump healthy seeds, but we don't expect a dent corn as the next year's result. We very properly endeavor to give our potatoes a balanced ration, in expectancy of a larger yield of well-matured, healthy tubers, but we should not expect these tubers to affect our next season's supply other than by their health. Similarly we take scions from well-lighted parts of the tree where growth has been good. In such twigs the graft union heals easily and properly, and a fit channel for conveying nutrients is established. In doing these things we are practising sanitation or preventive medicine, as it were, a laudable proceeding. But the horticulturist who promises a *different variety* by such means is illogical and misleading.

Yet we find Bailey so imbued with the idea of making out a perfect case for Lamarckism that he lends the weight of his authority to the following statement among others:[2]

> Whilst these "sports" are well known to horticulturists they are generally considered to be rare, but nothing can be farther from the truth. As a matter of fact, every branch of a tree is different from every other branch, and when the difference is sufficient to attract attention, or to have commercial value, it is propagated and called a "sport."

We may admit the differences between the branches of a tree without cavil. What is more serious is the impli-

[2] "Survival of the Unlike," p. 72.

cation to the reader that all variations have the same coefficients of heredity, that a bud-variation is simply a wide fluctuation imposed by external conditions. If this were true the whole organic world would be chaos. But species and varieties do exist. They may be "judgments" in one sense, but in another they are concrete things. In fact we learn this further on in this volume when it suits Bailey's purpose to have asexually propagated varieties very constant. He says (p. 353):

> **At first thought this fact—that varieties may be self-sterile—looks strange, but it is after all what we should expect, because any variety of tree fruits, being propagated by buds, is really but a multiplication of one original plant, and all the trees which spring from this original are expected to reproduce its characters.**

3. *The Effects of Disease.*—The influence of disease is in many ways like that of malnutrition, in that it is wholly an effect on the physiological efficiency of the reproducing cells. This fact is fairly clear when dealing with diseases with outstanding symptoms. In many instances, however, diseases are not easily diagnosed. There may even be no suspicion that disease is present. In such cases it is rather hard to believe that selection is not accomplishing a positive and radical improvement. A good example of this is the selection of potato tubers. No one consciously selects a seed potato infected with blight. Independent of the probability of reinfection, there is the likelihood that the diseased tuber will not be able to produce a normal plant because of the effect the fungus has had on its own cells. One doesn't usually believe, however, that rejection of this tuber and selection of the healthy sister is going to lead to the formation of a new race. Yet numerous experiments on potatoes in which it is shown that successive selections have raised the average yield over that of the unselected tubers, are probably of just this type. The race is kept up by the rejection of diseased tubers, but there is no evidence whatever that it is *improved*. I am not going to argue that desirable asexual variations may not occur during this time, and be retained. I say only that any improvement

indicated by the raw data must be discounted by the amount of deterioration shown by the unselected variety under similar conditions. Such deterioration is very common, and is due to disease, I believe, rather than to any supposed disadvantage of asexual reproduction *per se*.

This category of facts has been cited under the discussion of the inheritance of acquired characters, because such phenomena have perplexed other than botanists. Belief in the transmission of disease, or the effects of disease, by sexual reproduction was current for many years. It is only since the possibility of infection in the egg itself was demonstrated for various diseases, that the true state of affairs has been known.

Many other types of experiments designed to demonstrate Lamarckism might be cited, but they have no direct bearing on bud-variation except in so far as a positive case would affect our general attitude on the frequency of their occurrence. They are all similarly negative or questionable, however, so that we must conclude with Weismann that no case of inheritance of acquirements has been proved beyond a reasonable doubt. In other words we grant such a possibility but believe it to be so rare or so gradual that practically it may be disregarded.

In reality one could hardly have expected any other conclusion from the type of experiment by which the question has been attacked. Generalized they are something like this. Species *X* having been grown under environment *A* for numerous generations is removed to environment *B*. An adaptive change occurs which persists during several generations. Later the descendants of the original plants are returned to environment *A* and the change is reversed. When the reverse change occurs more slowly than the original change, it is argued that Lamarckian inheritance is shown. The logic used to draw such a conclusion is indefensible, even if the difficulty of correcting properly for changes due to normal heredity is left out of consideration.

If acquired characters are inherited and the changes

induced are reversible, the long period under environment *A* should have produced a deep impression on species *X*. Change under environment *B* should be slow. Reversal should be rapid, however, because of the slight impression environment *B* must be supposed to have made during the very few generations in which its influence was possible.

If acquired characters *are not inherited*, precisely the same changes should occur, owing to somatic adaptation, the only differences being that the total amount of change in each case would be reached in the second generation after the environment had acted during the earliest stages of the life history.

If, on the other hand, the changes induced by environment *B* are not reversible, judgment must be based on the percentage of individuals changed by *B* and not rechanged by *A*. One can readily see how a just judgment would be clouded by probable reversible somatic effects in such cases. Instances of the inheritance of acquirements, unless they were very frequent, which from our general evidence is unthinkable, would be indistinguishable from ordinary chance variations.

Such methods of attack on the subject being almost predestined to failure from the inherent difficulties of the problem, it would seem wiser to seek for a more hopeful methodology, and in the meantime to accept the only conclusion justified by the data at hand; namely, the inheritance of acquired characters is either so rare an occurrence or so slow a process, that by plant-breeders it may be assumed to be non-existent. One realizes of course that the problem of sexual transmission of somatic acquirements is not necessarily the same as that of asexual transmission, but the experimental results have been the same in both cases. Let us, admit, therefore, that one can not hope to obtain real improvement in asexually propagated varieties merely by selecting buds from plants or parts of plants which have developed under especially favorable conditions.

This does not mean that radical environmental changes

may not be the direct cause of such a modification. Dr. H. J. Webber once informed the writer that immediately after the great Florida freeze of the early nineties bud-variations in the citrus fruits of that region were greatly increased. Such variations may have been induced by the freezing, but they were not adaptive variations.

The conclusions reached thus far have not involved a point of theory which practically is difficult to separate from the one just discussed. It is this. If we disregard adaptive variations, is there not still a reason for selecting fluctuations? Are there not internal factors which so act that there is a narrow but appreciable variability in an asexually produced population which may offer a basis for selection? In other words, how constant is an asexually propagated race?

We can make an effort to compute the frequency of marked bud-variations. But have we any right to assume that these represent the sum total of all bud-variations? Are not bud-variations and perhaps all inherited variations like residual errors, the small ones frequent, the large ones rare? This may be the case, but I should like to emphasize the fact that we have no true criterion for determining the size of a variation. A variation that appears large by visual criteria may be an extremely small change in the constitution of the plant, and *vice versa*. In view of this fact together with the practical consideration that commercially valuable variations must be measurable within a reasonable duration of time—say a lifetime—it is by no means certain that we are going far astray in calculating the frequency of bud-variations by the so-called marked jumps or mutations.

Furthermore the range of the fluctuations of asexually propagated varieties of most species is very small even when broadened—as it always is—by the addition of the effects of variable external conditions. It is not hard to recognize a Winesap apple, a Clapp's Favorite pear or a Concord grape, even though these varieties have been grown extensively for a considerable number of years. Certain local subvarieties of the pome fruits are said to

exist, but they are so extremely rare that one may admit all cases of disputed origin and still have very little asexual variation to account for.

I have never seen a published calculation of the frequency of bud-variation, and presume it would be of little value anyway, since the general evidence indicates a different frequency for different species and even for the same species at different times. It may be mentioned, however, that in personal examination of over 100,000 hills of potatoes belonging to several hundred varieties, 12 definite bud-variations have been seen, a frequency of 1 in 10,000; while just as careful a scrutiny of about 200,000 plants belonging to the genus *Nicotiana* has brought to light but 1 case.

Probably a more practical and just as satisfactory an estimate of the frequency of bud-variations in economic plants is the record of varieties that have been produced in this manner. Naturally such a record contributes little to theory because only a portion of the variations arising are observed, and only a fraction of those observed are propagated. Further, the origin of comparatively few commercial varieties is known. Yet we may get some idea of what to expect in the future, by noting what has occurred in the past.

Data gathered in this manner will appear to give us different values depending on how we approach the matter. For example, in Cramer's wonderful monograph on bud-variation, the grape is cited as one of the species that often varies in this manner. He cites some 25 or more such varieties. Yet in the large list of American grapes in Hedrick's "Grapes of New York" only one doubtful case of bud-origin is reported. When one remembers that hundreds of varieties of grapes are grown and millions of vines are examined each year, improvement by this method seems rather hopeless. And examination of the list of present-day apples, pears, plums and cherries, of the bush-fruits, or of potatoes—all groups of considerable horticultural importance—is still more disappointing, for I venture to say that the varieties of

these types in cultivation which have originated as bud-variations can be counted on the fingers of one hand.

At the same time it would be wrong not to attribute any importance to bud-variation as a plant breeding adjunct. Cramer lists several hundred chrysanthemums and over a hundred roses as of bud-origin, as well as a smaller number of varieties in species where bud-variation appears to be less prevalent. Further, Shamel is said to have found bud-variation in the citrus-fruits to be sufficiently common to be worthy of an extended investigation.

These species, however, with perhaps the banana and the pineapple—the origin of whose varieties is little known—are the outstanding examples of comparatively frequent bud-variation, picked from our whole long list of cultivated plants. The first two examples, moreover, are species belonging to the domain of floriculture, where rather superficial characters such as color are valuable. In very few other species have bud-variations been recorded in sufficient numbers to justify us in employing any other adjective than "rare" in describing them. And of the sum total of these varieties only an extremely small percentage are of such a nature that agriculture would suffer a material loss if they were eliminated.

Perhaps these last statements appear to imply a very limited type of bud-variations. This is not true. Bud-variations are wholly comparable to seed-variations in their nature, but they are handicapped because recombinations of variant characters are possible only in sexual reproduction. *N* bud-variations in a species are simply *N* variations, but *N* seed-variations may become 2^n seed-variations provided they are not linked together in heredity. An immense advantage thus accrues in favor of seminal reproduction because by far the greater number of commercially valuable characters are complex in their heredity, *i. e.*, they are represented in the germ-plasm by several factors independently inherited.

Cramer divides bud-variations into the same classes that de Vries has used for sexual mutations: progressive,

where new characters arise; retrogressive, where a character becomes latent or lost; and degressive, where latent characters become active. In this important monograph practically all recorded bud-variations to the date of publication, 1907, are discussed. Yet not a single case of *progressive* variation is listed. They are all catalogued as retrogressive or degressive. Their classification is correct, however, only when a progressive variation is defined as the addition of a character wholly unknown in the previous history of the species.

As examples of what bud-variation does produce we may well study Cramer's painstaking work. There are losses of thorns, hairs and other epidermal characters, together with an occasional degressive change of the same kind. There are changes in color in vegetative parts. Green becomes red or "aurea" yellow, or a loss of anthocyan occurs. Sometimes the changes are such that the plants remain striped or otherwise variegated. Flowers and fruits exhibit the same types of color variations in considerable numbers. They are mostly losses, with the appearance of what in Mendelian terminology is called hypostatic colors, but once in a great while epistatic colors recur anew.

Monstrosities appear. Other parts of the flower take on the appearance and form of petals or of sepals. Doubling occurs in several different ways. Fasciations arise. Changes in the character of the reproductive apparatus are not uncommon, sometimes giving us seedless fruits.

Plants change their habit of growth. They become dwarf. They retain juvenile characters. They become laciniate, or develop the trait known as "weeping."

Thus we see that bud-variation is not limited in its manifestations; and what is more important, we realize that bud-variations are very comparable to seminal variations, there being hardly a type of change known in sexually reproduced plants that has not been duplicated asexually. What then is the difference, if any, between true somatic changes and true germinal changes in constitution? We can get clues which indicate a fairly satis-

factory solution of this problem from three different lines of research, pedigree cultures, graft-hybrids and cell-studies.

It is a noteworthy fact that the character of the progeny produced sexually by bud-variations has been studied in a comparatively few cases, and in most of these instances self-pollinations were not made. Nevertheless Cramer believes the following conclusions are justified:

1. In a vegetative Mendelization, of the progeny of a branch with the positive character 75 per cent. have the character and 25 per cent. are without it, while the progeny of a branch without the character all lack it.

2. In a vegetative "Zwischenrasse" by which he generally means a variegated race, of the progeny of each type (original and variant), a part retain and a part lack the character, the percentage being variable.

3. In a vegetative mutation, by which he means any change not a "Zwischenrasse" and which did not appear to him to be Mendelian in type, of the progeny of a branch retaining the positive character, either all possessed it or a part were with and a part without it, while the progeny of a branch without the character were all of the same type.

If we allow for some deviation due to cross-pollination, I believe that Cramer's records support this view, and that modern genetic research suggests the interpretation.

In the first place, the "Zwischenrasse" are evidently of the type studied principally by Correns and by Baur in sexually reproducing races. They are due to chromatophore changes, and in many cases at least are not the result of nuclear activity. This being true, one would expect in neither asexual nor sexual reproduction the same type of inheritance for variegated races that obtains for other types of variation. Inheritance will parallel cytoplasmic rather than nuclear distribution; an expectation apparently realized for both types of reproduction.

Omitting the "Zwischenrassen" therefore, we have two phenomena to explain, both of which are similar to cases of inheritance in sexual reproduction where chromatin

distribution parallels the facts. In each instance the negative variant—may we call it the recessive—breeds true. In one case the positive variant breeds true, in the other case it gives a simple Mendelian ratio.

The mechanism necessary for such phenomena is not difficult to picture. Bud-variations are many times more frequent in hybrids, that is, in plants heterozygous for one or more characters, than they are in *pure* species. This is the view of Cramer, this was the view of Masters, the eminent English student of bud-variations and teratological phenomena, this was the conclusion drawn by the present writer in several articles published some years ago. Such results would be obtained either when the proper germinal change occurs in the chromosome whose mate lacks a character for which the plant is heterozygous; or, when there is a dichotomy in which the chromosomes of such a pair are not halved but pass the material basis necessary for the production of the positive character to one daughter cell and not to the other, provided the daughter cell lacking the character gives rise to a branch.

A bud-variation in a character for which the plant was homozygous would be obtained only when simultaneous like changes occur in both chromosomes of a homologous pair, or when the material basis necessary for the production of the positive character all passes to one daughter cell, as described above.

This hypothesis would account for the fact that heterozygotes give rise to bud-variations more frequently than homozygotes, since a germinal change seldom gives rise to a new positive character, and a change in one chromosome of an identical pair tending toward the production of a recessive, would not show in the latter case.

I am not certain that this hypothesis may not with reason be applied to variations that are usually considered seminal. There is no particular ground for assuming that such variations occur only at the maturation of the germ-cells. We know that progressive variations of whatever origin are extremely rare. Why then may not

most variations be produced in cell divisions previous to the formation of the germ-cells? When recessive we should not note them as bud-variations unless the plant is heterozygous and the mutating cell gives rise to a branch; when dominant we should only note them in the latter eventuality. But if these mutating cells should later give rise to germ-cells, the change would become apparent in the progeny.

We have still one other hypothetical case to consider. It is said that some bud-variations are not transmitted by seed. I have not been able to trace an authentic case, but such is the general belief, fathered, I think, by Darwin. The usual citation is the nectarine, which sometimes is said to give nectarines but at other times gives only peaches. Whether trichome characters only behave thus I do not know. But if that be true, we can understand why if we refer to Winkler's work on the so-called graft-hybrids.

Winkler found that the most interesting of these peculiar phenomena are caused by the tissue of one species growing around the tissue of the other. He therefore gave them the euphonious name of periclinal chimeras. Cytological examination showed that the epidermal tissues only are from one race, the remaining tissues being from the other. It is really a symbiosis and not a union. Now as the germ-cells are formed wholly from subepidermal and never from epidermal tissues, the seeds of these plants always produced seedlings like the type forming the *inner cell-layers*.

It seems probable that the production of the nectarine may be analogous. If the change producing the nectarine occurs after the epidermal tissue has been segregated from other tissues, the cells which are ancestors of the germ-cells should not be affected and the nectarine seedlings would give peaches. If, on the other hand, the change producing the nectarine, has occurred before any such segregation, the progeny sexually produced should in part be nectarines.

THE PROBABLE ERROR OF A MENDELIAN CLASS FREQUENCY[1]

DR. RAYMOND PEARL

1. With the increasing volume of Mendelian experimentation there is an ever-growing need for adequate and clearly understood tests for the statistical significance of differences between observed results and expectation. A number of different methods of making such tests have been proposed and used by different workers. For example, early in the discussion of Mendelism, Weldon[2] made use of the ordinary, and frequently inadequate, expression for the standard deviation of a sub-frequency $\sigma = \sqrt{npq}$. Johannsen[3] has also made much use of this method. It has several defects. In the first place it assumes the Gaussian distribution of the errors, an assumption not often strictly warranted, as Pearson[4] has clearly shown, and in many cases grossly in error. In the second place it is not even approximately adequate under certain extreme conditions (frequently realized in Mendelian work) of class frequency. Harris[5] has proposed the χ^2 "goodness of fit" test for comparing observed and expected Mendelian distributions. There are several features of this method which greatly limit it for such use. Among these are (1) its failure to make correct allowance for "tail" frequencies (it is just this class of very small frequencies which one most often wants to test in prac-

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 108. The author is greatly indebted to his assistant, Mr. John Rice Miner, for the laborious arithmetic involved in Section 6 of the paper.

[2] Weldon, W. F. R., "Mendel's Laws of Alternative Inheritance in Peas," *Biometrika,* Vol. I, pp. 228–265, 1902.

[3] Johannsen, W., "Elemente der exakten Erblichkeitslehre," Zweite Ausgabe, Jena, 1913.

[4] Pearson, K., "On the Influence of Past Experience on Future Expectation," *Phil. Mag.,* March, 1907, pp. 365–378.

[5] Harris, J. A., "A Simple Test of the Goodness of Fit of Mendelian Ratios," Amer. Nat., Vol. XLVI, pp. 741–745, 1912. *Cf.* also Pearson, K., and Heron, D., "On Theories of Association," *Biometrika,* Vol. IX, pp. 159–315, 1913.

tical Mendelian work), and (2) the fact that the test takes into account only the *magnitude* of the error and not its *direction* (*i. e.*, whether in excess or defect) in any particular case. (3) It gives a result not particularly well adapted to the actual needs of Mendelian research. The χ^2 test gives a measure of the goodness of fit of the *whole* distribution, and *only* that. Now besides being interested in that point the Mendelian worker quite as often wants to know, in addition, something about the probability that *particular classes* observed are significantly different from the expected. To that sort of knowledge the χ^2 test helps him not at all. It is an "all or none" sort of method.[6]

2. It has seemed to the writer that it would be useful to discuss methods of determining the *true* probable error of each class frequency in Mendelian distributions as a supplement to the χ^2 test, and for use in cases where it is not applicable. The fundamental theorems have all been given by Pearson[7] in a very important paper, which is apparently almost entirely unknown to biologists. The purpose of the present paper is first to show the applicability of these theorems to the problem in hand, and second to point out some matters regarding the practical use of the method likely to be helpful to biologists with but little mathematical training who may attempt to use it.

3. In the paper referred to, Pearson, starting from Bayes' theorem, shows that the distribution of chances of an event occurring in a particular way in a second

[6] I have earlier pointed out other objections to the χ^2 test in Mendelian work, in particular its total failure to deal with cases where experiment yields a small frequency on classes where the expectation is zero, and need not further discuss them here. I have never thought it necessary to make any rejoinder to Pearson's characteristically bitter reply to my criticism, nor do I yet. The χ^2 test leads to this absurdity: if I perform a Mendelian experiment in which I get ten thousand million offspring agreeing *perfectly* with expectation save for *one* lone individual (perhaps a mutation, perhaps a mistake in the record, or what not) which is of a sort not expected, then Pearson and the χ^2 test agree that the probability is infinitely great that the ten thousand million offspring *do not* follow Mendelian law!

[7] Pearson, K., *loc. cit.*

sample from a population from which a first sample has produced a certain value is given, *not* by the ordinates of a normal curve of errors, as is commonly assumed in writings on the theory of probability, but by the successive terms of a simple hypergeometrical series. In an earlier paper the same author[8] had solved the problem of the momental properties of the hypergeometrical series. Combining the two results he was able to derive the necessary equations for the complete solution of the problem of probable errors in sampling. We may proceed at once to the exposition of these results, referring the reader for the proofs to the papers of Pearson cited.

Let it be supposed that a first sample of $n = p + q$ be drawn from the population, p denoting the number of times the event dealt with occurs in the n trials, and q the number of times it fails.

Write

$$\bar{p} = \frac{p}{n}, \quad \bar{q} = \frac{q}{n},$$

whence of course

$$\bar{p} + \bar{q} = 1.$$

We then have for the chief constants of the error distribution for a *second sample*, of magnitude m, drawn from the same population the following values:

$$\text{Mean}^9 = m\bar{p} + \frac{m}{n+2}(\bar{q} - \bar{p}), \tag{i}$$

$$\text{Mode} = \text{the } \textit{integral} \text{ portion of } mp + p, \tag{ii}$$

$$\text{Standard Deviation} = \left\{ m\left(\bar{p} + \frac{\bar{q} - \bar{p}}{n+2}\right) \times \left(q - \frac{\bar{q} - \bar{p}}{n+2}\right)\left(1 + \frac{m-1}{n+3}\right)\right\}^{\frac{1}{2}}. \tag{iii}$$

These values are entirely general, and independent of the values of n, m, p and q. Under certain circumstances,

[8] Pearson, K., "On Certain Properties of the Hypergeometrical Series, and on the Fitting of such Series to Observation Polygons on the Theory of Chance," *Phil. Mag.*, Feb., 1899, pp. 236–246.

[9] From origin at the lower range end, or $r = 0$.

as when n is very large as compared with m, and neither p nor q are very small, (i) and (iii) are obviously capable of being put in much simpler form and still giving a sufficiently close approximation to the true result. For Mendelian work, however, where frequently neither of these conditions are even approximately realized, it will in general be better to use the full expression as given above.

The ordinates of the error distribution (the chances of different occurrences) are given by the successive terms of the hypergeometrical series

$$C_r = C_0 \left\{ 1 + \frac{m}{1} \cdot \frac{p+1}{q+m} + \frac{m(m-1)}{2} \cdot \frac{(p+1)(p+2)}{(q+m)(q+m-1)} + \frac{m(m-1)(m-2)}{\lfloor 3} \cdot \frac{(p+1)(p+2)(p+3)}{(q+m)(q+m-1)(q+m-2)} + \frac{m(m-1)(m-2)(m-3)}{\lfloor 4} \times \frac{(p+1)(p+2)(p+3)(p+4)}{(q+m)(q+m-1)(q+m-2)(q+m-3)} + \text{etc.} \right\}, \tag{iv}$$

where

$$C_0 = \frac{\Gamma(q+m+1)\Gamma(n+2)}{\Gamma(q+1)\Gamma(n+m+2)}. \tag{v}$$

As we shall presently see, the calculation of the terms in (iv) becomes a tedious and laborious matter when the number needed is at all considerable. Under such circumstances, and when in addition m and n are even moderately large, equation (iv) may be greatly simplified, without significant loss of accuracy, by the use of Sterling's theorem (to the bracket) or by Forsyth's approximation for such of the factorials as are not included in the range of the Pearson[10] tables. Thus we have, by Sterling's theorem, remembering that r denotes any term in the series, and writing $s = m - r$,

[10] "Tables for Statisticians and Biometricians," edited by Karl Pearson, Cambridge, 1914.

$$C_r = C_0 \frac{\lfloor p+r}{\lfloor p \lfloor r} \left\{ \frac{m^{m+\frac{1}{2}}(q+s)^{q+s+\frac{1}{2}}}{(q+m)^{q+m+\frac{1}{2}} s^{s+\frac{1}{2}}} \right\}. \quad \text{(vi)}$$

Using Forsyth's approximation, which is extremely accurate, one gets

$$C_r = C_0 \frac{\lfloor p+r}{\lfloor p \lfloor r} \left\{ \frac{(m^2+m+\frac{1}{6})^{m+\frac{1}{2}}\{(q+s)^2+(q+s)+\frac{1}{6}\}^{q+s+\frac{1}{2}}}{(s^2+s+\frac{1}{6})^{s+\frac{1}{2}}\{(q+m)^2+(q+m)+\frac{1}{6}\}^{q+m+\frac{1}{2}}} \right\}^{\frac{1}{2}}. \quad \text{(vii)}$$

The gamma terms in (v) will, of course, be calculated by some one of the well-known approximations (*e. g.*, Sterling's, Pearson's, Forsyth's) or by interpolation from a table of factorials (Pearl[11]).

4. The proposal which I wish to make for the expression of a Mendelian result is that *the expectation be expressed as the quartile limits for each class frequency in a second sample of the same size as the observed sample.* In using such an expression it must be clearly understood that it does *not* measure the goodness of fit of the distribution *as a whole,* because it takes no account of correlations in errors. What it does give, in supplement to the χ^2 test, is the limits of probability of each class frequency, *taken by itself.*

The ordinary expression for a probable error (*e. g.*, P. E. mean $= \pm .67449(\sigma/\sqrt{n})$) gives the quartile limits (*i. e.*, the limits within which one half the frequency occurs) on the assumption that the distribution is Gaussian, since in such a distribution of unit area the quartile limits are .6744898 . . . times the standard deviation on either side of the mean. But in our persent work we are making no assumption that the error distribution is Gaussian. Consequently we must determine the quartiles directly from the distribution. In cases where the number of terms is not too great the ordinates may be calculated from (iv) or (vi) and summed to find the quartile. In many cases, however, this would be practically too tedious

[11] Pearl, R., "Interpolation as a Means of Approximation to the Gamma Function for High Values of *n*," *Science,* N. S., Vol. XLI, pp. 506–507, 1915; "On the Degree of Exactness of the Gamma Function Necessary in Curve Fitting," *Ibid.,* Vol. XLII, pp. 833–834, 1915.

an operation, and we may resort to an approximate method. The simplest one is to take $.67449\sigma$ on either side of the median, which is approximately determined by remembering that the median lies between the mean and the mode and approximately twice as far from the mode as from the mean. The criterion of whether this method of fixing the quartile limits may be safely applied will be found in the value of the skewness, *Sk*. In practical work this approximate method will give sufficiently accurate results unless the skewness is very large, say > 0.6.

We have by definition

$$Sk = \frac{d}{\sigma}. \qquad \text{(viii)}$$

Hence having calculated the values of mean, mode and σ by (i), (ii) and (iii) we can readily obtain (viii), since $d =$ mean $-$ mode.

We may now pass to the consideration of some numerical examples, by means of which certain facts can be better brought out than by further theoretical discussion.

5. As a first and simple example we may take some data, recently published by F. L. Platt,[12] on the results of mating Blue Andalusian fowls. On account of the frequency with which the Blue Andalusian case is cited as a paradigm in Mendelism, coupled with the great dearth in the literature of exact statistics of actual matings of this breed of poultry, it seems especially worth while to discuss these statistics furnished by Mr. Platt, on the authority of Mr. W. J. Coates, a breeder of Andalusians.

Table I gives the data, and in the last line, the Mendelian expectation expressed in the form suggested in this paper.

The occurrence of the "dark reds," which Mr. Coates informs us had a pattern like a Red Game, is a phenomenon not mentioned in textbook accounts of Mendelian inheritance in the Blue Andalusian. In the present con-

[12] Platt, F. L., "Western Notes and Comment," *Reliable Poultry Journal*, Vol. XXIII, p. 665, 1916.

TABLE I

SHOWING THE RESULT OF MATING BLUE × BLUE IN THE BLUE ANDALUSIAN BREED OF POULTRY (COATES' DATA)

Mating	Offspring			
	White	Blue	Black	Dark Red
A	4	10	3	1
B	4	5	2	0
C	3	3	0	3
D	0	12	1	0
E	3	3	1	0
Total observed	14	33	7	4
Total observed in categories (1) white, (2) blue, (3) pigmented not blue	14	33	11	
If the true ratio is 1 white : 2 blue : 1 pigmented not blue it is an even chance, considering each class by itself, that the frequencies in a sample of this size will fall between	*11.5 and 17.8*	*25.8 and 32.9*	*11.5 and 17.8*	

nection, however, we can not pursue that point, but will group together, as in the penultimate line of the table, the blacks and reds as "pigmented, not blue," and assume that the three classes should occur in a 1:2:1 ratio. Do the actual results bear out this assumption, having regard to the errors of sampling?

Examining the last two lines of the table, it is clear that each observed class, taken by itself, is by no means an impossible approximation to what would be demanded by a 1:2:1 ratio. The blues and the "pigmented not blues" fall outside the range for which the probability is $\frac{1}{2}$ but only slightly outside. It would be practically an even bet, if Blue Andalusians really follow a law of 1:2:1 segregation when bred together, that any particular sample of 58 offspring would show in each particular class as great a deviation as the present sample.[13]

Now we may consider in detail the mode of calculating the figures in the last line of Table I.

[13] Always on the assumption, of course, that it is legitimate to lump the blacks and reds together. There is room for scepticism on that point, but we are here only concerned with the case as an illustration of method.

We have, by hypothesis, and from the statistics

$$m = n = 58.$$

A distribution of 58 in a 1:2:1 ratio is 14.5:29:14.5. Assume a first sample of 58 to show exactly this distribution 14.5:29:14.5, what will be the mean frequency of one of the end classes, say white, expected in a second sample of 58?

We have

$$\bar{p} = \frac{14.5}{58} = .25,$$

$$\bar{q} = .75,$$

whence, by (i),

$$\text{Mean} = 14.9833,$$

and, by (ii),

$$\text{Mode} = 14,$$

$$d = \quad .9833.$$

By the approximate method we get

$$\text{Median} = 14.656 \text{ approximately.}$$

The standard deviation from (iii), is

$$\sigma = 4.6364,$$

and, by (viii),

$$Sk. = \quad .2121.$$

Actual tests with curves of a degree of skewness no greater than this show that the approximate method gives the quartile limits with sufficient accuracy for practical purposes. We have for the approximate quartile limits, $.67449 \times 4.6364 = 3.1272$. This value, added to and subtracted from the median 14.656, gives the results set down in the last line of Table I.

Exactly the same procedure, with different numbers, is followed in the case of the blue column.

6. Let us now consider a more completely worked out illustration. Some time ago Mr. Alexander Weinstein, of Columbia University, consulted the writer in regard to a

problem which had arisen in connection with his Mendelian breeding experiments. A certain type of mating gave the following class frequencies:

$$6363 + 579 + 3638 + 1208 + 128 + \overset{(x)}{115}$$
$$+ 350 + 6 = 12387 \quad . \quad . \quad . \quad (a).$$

Another group of matings gave a total of 9,017 offspring, of which 30 fell in the x class, this being the only class in regard to which a comparison is to be made. On certain theoretical grounds the percentage frequency in this x class in the second sample would be expected to be 0.582 times the percentage frequency of this same class in the first sample. The question is whether the actually observed frequency of 30 in this second sample is such as could reasonably be expected to occur if the theoretical assumption actually were the fact.

It will be seen at once that, owing to the very small absolute frequency of this x class in both samples, ordinary probable error methods will be of no avail.

Approaching the problem by the method here proposed, we have, as basic values for the computations,

$$n = 12387$$
$$m = 9017$$
$$p = 115,\ q = 12272$$
$$p = p/n = .009284$$
$$q = q/n = .990716.$$

Whence we have for the mean in the second sample of 9017 by (i)

$$\text{Mean} = 84.428137$$

and by (iii)

$$\sigma = 12.020652.$$

By (ii) the $mode = 83$, whence $d = 1.428137$

and

$$sk = \frac{1.428137}{12.020652} = 0.118807.$$

Working directly from the moments of the hypergeometrical series and, in effect, replacing that series with a true curve, we find

$$\text{Mode} = 83.222141,$$
$$d = 1.205996, \text{ and}$$
$$Sk = 0.100327 \pm .008696.$$

TABLE II

SHOWING THE SUCCESSIVE ORDINATES OF THE HYPERGEOMETRICAL SERIES FOR THE SECOND SAMPLE

r	C_r	*Sum*	r	C_r	*Sum*	r	C_r	*Sum*
35	.000000	.000000	73	.022620	.182838	111	.003215	.983125
36	.000001	.000001	74	.024355	.207193	112	.002740	.985865
37	.000001	.000002	75	.025996	.233189	113	.002325	.988190
38	.000002	.000004	76	.027539	.260728	114	.001964	.990154
39	.000003	.000007	77	.028943	.289671	115	.001651	.991805
40	.000005	.000012	78	.030183	.319854	116	.001382	.993187
41	.000008	.000020	79	.031236	.351090	117	.001152	.994339
42	.000013	.000033	80	.032085	.383175	118	.000957	.995296
43	.000020	.000053	81	.032715	.415890	119	.000791	.996087
44	.000031	.000084	82	.033116	.449006	120	.000651	.996738
45	.000046	.000130	83	.033285	.482291	121	.000533	.997271
46	.000068	.000198	84	.033220	.515511	122	.000436	.997707
47	.000099	.000297	85	.032929	.548440	123	.000354	.998061
48	.000142	.000439	86	.032419	.580859	124	.000287	.998348
49	.000200	.000639	87	.031706	.612565	125	.000231	.998579
50	.000279	.000918	88	.030805	.643370	126	.000186	.998765
51	.000383	.001301	89	.029738	.673108	127	.000149	.998914
52	.000520	.001821	90	.028526	.701634	128	.000119	.999033
53	.000696	.002517	91	.027193	.728827	129	.000094	.999127
54	.000919	.003436	92	.025763	.754590	130	.000075	.999202
55	.001199	.004635	93	.024262	.778852	131	.000059	.999261
56	.001545	.006180	94	.022711	.801563	132	.000046	.999307
57	.001967	.008147	95	.021136	.822699	133	.000036	.999343
58	.002476	.010623	96	.019556	.842255	134	.000028	.999371
59	.003082	.013705	97	.017991	.860246	135	.000022	.999393
60	.003793	.017498	98	.016459	.876705	136	.000017	.999410
61	.004617	.022115	99	.014974	.891679	137	.000013	.999423
62	.005561	.027676	100	.013550	.905229	138	.000010	.999433
63	.006629	.034305	101	.012195	.917424	139	.000008	.999441
64	.007821	.042126	102	.010917	.928341	140	.000006	.999447
65	.009135	.051261	103	.009722	.938063	141	.000005	.999452
66	.010569	.061830	104	.008614	.946677	142	.000004	.999456
67	.012109	.073939	105	.007593	.954270	143	.000003	.999459
68	.013743	.087682	106	.006660	.960930	144	.000002	.999461
69	.015455	.103137	107	.005813	.966743	145	.000001	.999462
70	.017223	.120360	108	.005049	.971792	146	.000001	.999463
71	.019025	.139385	109	.004364	.976156	147	.000001	.999464
72	.020833	.160218	110	.003754	.979910			

The two sets of values are evidently sufficiently near together to be used interchangeably for most practical

purposes, a sort of result which is familiar to any one who has had any considerable experience with the method of moments.

To return now to the series we find, using 10-place logarithms in the intermediate computations and the Forsyth approximation,

$$\log C_o = 72.3493814 - 100.$$

We have now to calculate the successive terms of the series. If this were done for the whole range it would involve a literally colossal amount of labor. Fortunately this is not necessary. We need only take that part of the range which includes appreciable frequencies. By a few trials we find that this part of the range begins with $r = 36$. In Table II are given the frequencies for the several terms in the series between $r = 36$ and $r = 147$ inclusive, the total area being taken as unity. To reduce these frequencies to the actual numbers for the second sample we have only to multiply in every case by 9017. We have calculated C_{36} by (vii) and used the Forsyth C_o.

From this table we easily deduce

$$\begin{aligned} \text{Median} &= 83.5331, \\ \text{Lower quartile} &= 75.6104 \\ \text{Upper quartile} &= 91.8218. \end{aligned} \qquad \text{(ix)}$$

Now, remembering that if the same law holds for the second Mendelian distribution as for the first we should expect the x class in that distribution to be 0.582 times the value of the same class in the first distribution, we have

Expected mean value of x class in second distribution = 49.14

Expected modal value of x class in second distribution = 48

Expected lower quartile value in second distribution = 44.01

Expected upper quartile value in second distribution = 53.44

The actual experimental value obtained was 30, which is far below the lower quartile. From Table II we find, remembering again that on *a priori* grounds the experimental frequencies are reduced by the factor 0.582, that if the two distributions were really samples of the same population, obeying the same Mendelian laws, it would be expected that the x class would show a frequency as low as or lower than 30, only 18 times in 10,000 trials of samples of 9017. Or, in other words, the odds against so low a value as 30 are about 556 to 1. These are about the same odds as those associated with the occurrence of a deviation 4.63 times the probable error (cf. Pearl and Miner[14]).

We may, therefore, conclude with great certainty that the value of 30 is significantly *smaller* than would be expected to occur in the x class on the basis of chance (deviation due to random sampling) if the two distributions were really samples of the same population.

Let us now go back and approach the problem *de novo* by the approximate method suggested in section 4. We have

$$d = \text{mean} - \text{mode} = 1.4281,$$

$$\frac{1.4281}{3} = .4760,$$

$$\begin{aligned} 84.4281 - \ .4760 &= 83.9521 = \text{median (approx.)}, \\ 12.0207 \times \ .67449 &= \ 8.1078, \\ 83.9521 - 8.1078 &= 75.8443 = \text{Lower quartile}, \\ 83.9521 + 8.1078 &= 92.0599 = \text{Upper quartile}. \end{aligned}$$

Comparing these values, in the obtaining of which all the tremendously tedious and time-consuming arithmetic involved in calculating Table II was avoided, with those shown in (ix) makes it quite evident that for all practical statistical purposes the approximate method would have given sufficiently accurate results.

[14] Pearl, R., and Miner, J. R., "A Table for Estimating the Probable Significance of Statistical Constants," Me. Agr. Expt. Stat. Ann. Rept. for 1914, pp. 85–88.

SUMMARY

In this paper is presented a method of calculating and expressing the errors, due to random sampling, of a Mendelian class frequency. The method consists essentially in expressing each *expected* Mendelian frequency as the probable quartile limits for that class frequency in a supposed second sample of the same size as the observed sample drawn from the same population. These quartile limits are determined from the ordinates of a hypergeometrical series. Various simplifications of method are suggested and illustrated. The method is suggested as a supplement to, not as a substitute for, the χ^2 test for the goodness of fit in Mendelian distributions.

OBSERVATIONS ON THE ECOLOGY OF THE PROTOZOA

By LEON AUGUSTUS HAUSMAN

Cornell University

A great deal of excellent work has been done in census taking among the Protozoa, and numerous catalogues of species with descriptions and accompanying plates have been issued for various states or portions thereof. This present communication, however, is a slight contribution toward census taking of a different sort, in that it aims to set forth some facts regarding the different types of Protozoan habitats and the species usually associated with each. It is well known that certain common forms, used as type species in the laboratory (such as *Amœba proteus* and *Paramœcium caudatum*), can be found only in certain fairly definite environments. The ecology of such forms is well known, but the bionomics of the majority of the Protozoa is still virtually a *res ignota*. The study of environmental units and Protozoan communities will contribute to a more intimate knowledge of these elusive forms.

Although little is definitely known concerning the ecology of the Protozoa, yet I think that we are in position to say that Protozoan inhabitants vary with their varying environments. A record of the inhabitants of a marsh pool will not include the same species normally found in clear running streams, nor will cold waters yield the same forms as warm. But we can go still farther, I think, and say that the various portions of any given environmental unit, even though they differ ever so slightly from one another, will each have its own characteristic group of organisms. The factors which are accountable for the variation in species or numbers of individuals of any species in such instances may be hardly discoverable, but they are none the less potent. Thus the entrance point of a tiny thread

of clear spring water in the midst of an impure pool may furnish an abundance of *Monas* and *Cercomonas*, and because of the presence of these, also the larger carnivorous forms such as *Dileptus* or *Onychodromus*. Or the growth of myriads of bacteria at another point may induce the increase in the numbers of *Vorticellæ*, *Amœbæ*, or *Holophryæ*. A complete study of the multitudinous minute variations in the environments of Protozoa, and their complex relationships with the associated species is still far away. But I believe that we are now ready to undertake a preliminary survey of a number of typical environments and to ascertain what genera and species may be normally expected to occur in each.

Of the many factors responsible for the localization of species, the following are perhaps first in importance: light, character of food, temperature of water, chemical content of water, presence or absence of enemies.

Light exerts a powerful influence in determining the distribution of species within a given body of water. Thus one will find certain species in sunlit areas and certain others in shaded ones. Not all of the Protozoa respond to ordinary light stimuli, as has been shown experimentally by Jennings.[1] He has found that comparatively few of the ciliate infusoria react to light stimuli. The flagellates, on the other hand, show a definite reaction, congregating in that region where the light is ordinarily strongest. The reactions of *Euglena viridis* and *Cryptomonas ovata* are the forms which have been most carefully studied in this regard.

Jennings[1] further showed that *Paramœcia* collect in those regions of the water which contain a trace of any weak acid, and this I have noted to be true of certain other ciliates. This may be a dominant influence in localizing forms in pools and streams in marshes where organic acids are usually present. The presence of supernormal amounts of oxygen, carbon dioxide, calcium carbonate, iron, and other dissolved substances may also affect the distribution of species.

[1] "Contributions to the Study of the Behavior of Lower Organisms," H. S. Jennings, Washington, 1904.

Not only may the various stimuli arising from the different elements of the environment assort species into communities, but the effects of these stimuli may not always be the same. A given stimulus may at one time produce a certain type of reaction, and at another time a somewhat different type. This fact, also, experimentally verified by Jennings, led him to suppose that something analogous to, if not identical with true varying "physiological states" obtains in the Protozoan body. If this be so then the problem of Protozoan ecology is still further complicated, as far as any assignment of species to definite fixed local environments is concerned.

For collecting Protozoa and water samples the writer has found the following useful: a small silk plankton net (with draw string bottom) about six inches in diameter and ten inches deep; a glass or metal pipette, fully a foot in length, operated by a compression bulb at one extremity, for sucking samples of light sediments from the bottoms of pools and streams; several small glass dipping tubes; a large table spoon; a thermometer, and a plentiful supply of variously sized water tight jars with screw tops. With such an outfit as this the material described in the following paper was secured. The samples were examined immediately after being brought into the laboratory, in order that the proportions of species might be accurately recorded.

From each sample ten slides were searched. The significance of the terms few, numerous, rare, etc., is as follows:

Over ten individuals of one species per slide .....abundant
Average of 5–10 .....numerous
Average of 2–5 .....several
Average of .5–2 .....few
Average of less than .5 .....rare

The samples were taken from the uppermost layer of silt on the bottoms of pools, streams, etc., the utmost care being taken not to disturb the sediment below or to roil the water. Upwards of fifty samples were secured representing five distinct environmental types. These five types described are:

No. 1. Characteristic marsh pools.
No. 2. Clear cold lakes and streams with no plant life.
No. 3. Clear springs and streams with plants abundant.
No. 4. Small, clear pools containing organic sediment in decomposition and fed by pure, cold rills.
No. 5. Ditches and pools choked with algæ, water warm.

All of the material was taken during the months of late spring, summer, and early fall, while the aquatic vegetation, and the semiaquatics about the water margins, were in vigorous growth.

ENVIRONMENTAL TYPE NO. I: MARSH POOLS

Characteristic frog-inhabited marsh pools, containing much decaying vegetable matter, supporting rank growths of typical marsh plants about the margins, covered with lily pads and filled with *Ceratophyllum, Myriophyllum, Sagittaria,* duckweed, etc. Water warm, and emitting characteristic swampy odor.

Predominant forms:

***Amœba limax,* several**
***Amœba proteus,* several**
***Amœba radiosa,* several**
***Arcella vulgaris,* numerous**
***Carchesium polypinum,* numerous**
***Codonocladium umbellatum,* numerous**
***Coleps hirtus,* abundant**
***Coleps* sp., abundant**
***Difflugia acuminata,* numerous**
***Difflugia corona,* numerous**
***Difflugia globulosa,* numerous**
***Euglypha alveolata,* numerous**
***Oikomonas* sp., numerous**
***Peridinium cinctum,* abundant**
***Stylonychia mytilus,* numerous**
***Stylonychia pustulata,* numerous**
***Stentor polymorphus,* numerous**
***Synura uvella,* numerous**
***Trinema acinus,* numerous**
***Vorticella microstoma,* numerous**
***Vorticella nutans,* numerous**

Other forms present in less numbers (few or rare):

Actinophrys sol
Arcella dentata
Arcella discoides
Anisonema obliqua
***Astasia* sp.**
Biomyxa vagans
Blepharisma lateritia
Centropyxis aculeata
***Colpoda* sp.**
Cothurnia maratima
Dactylosphærium radiosum
Dinomonas vorax
Euplotes carinata
Euplotes charon
Halteria grandinella
Heterophrys myriapoda
***Holophrya* sp.**
Lacrymaria olor
Lionotus wrzesmosky
***Lionotus* sp.**
***Loxophyllum* sp.**
Paramœcium bursaria
***Pleuronema* sp.**
Prorodon griseus

Stentor cæruleus
Trichodina pediculus (on *Hydra fusca*)
Urocentrum turbo
Volvox globator
Vorticella sp.

Blepharisma lateritia (Fig. 1) is usually described as showing pink or even reddish hues, and occurring rarely colorless. My experience has been to find the greater number of individuals colorless and a very few showing even a faint trace of pink. In reproducing the colors of Protozoa in plates there seems to be a tendency to represent them more vividly than they occur in nature. It is interesting to place a small chart of spectrum colors on the table near the base of the microscope and compare them with the hues of those species of Protozoa usually represented as brightly colored. It has been my experience to find that the (depicted) decided colors of some of the Protozoa show themselves to be only the faintest tints.

Synura uvella (Fig. 2) is not entirely colonial in its habit. I have found it sometimes singly, and often in pairs. The normal number of individuals in a colony is from 10 to 20. As many as 35 in one group have been recorded. The flagella are invisible unless iodine or some good stain has been used.

The *Coleps* sp. (Fig. 3) which I found so frequently in this one group of samples I have never found since. It was appreciably smaller than *Coleps hirtus* (Fig. 4), being about 30 microns in length, whereas *hirtus* is 50. *Hirtus* is a species exhibiting a notable constancy in dimensions, and this I found true also of all the numerous individuals of *Coleps* sp. which I measured. No transitions in size from one to the other could be found. *Coleps* sp. was not the result of fission on the part of *hirtus*, for in all the material my search revealed not a single individual undergoing division. In appearance and activities the one form was the exact counterpart of the other, with a solitary exception: the movements of *Coleps* sp. were at all times much more rapid than those of *hirtus*.

Lacrymaria olor (Fig. 5) described as a very variable species, is variable in *size* almost entirely. Its *form* is quite constant, and offers a virtually certain criterion for identification.

PLATE I

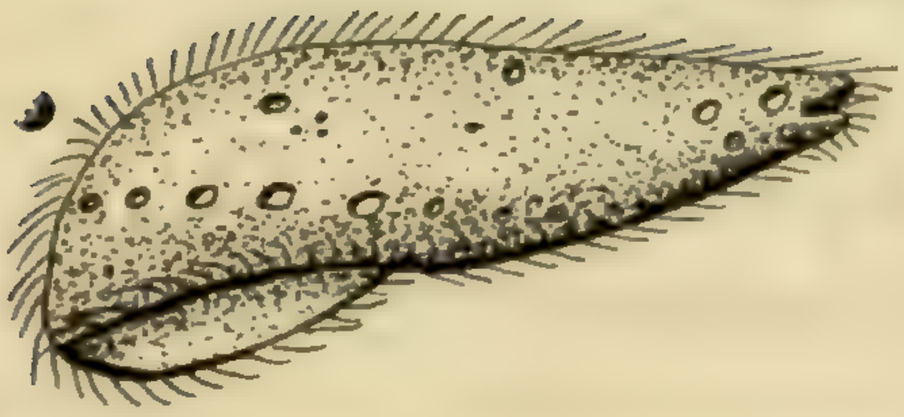
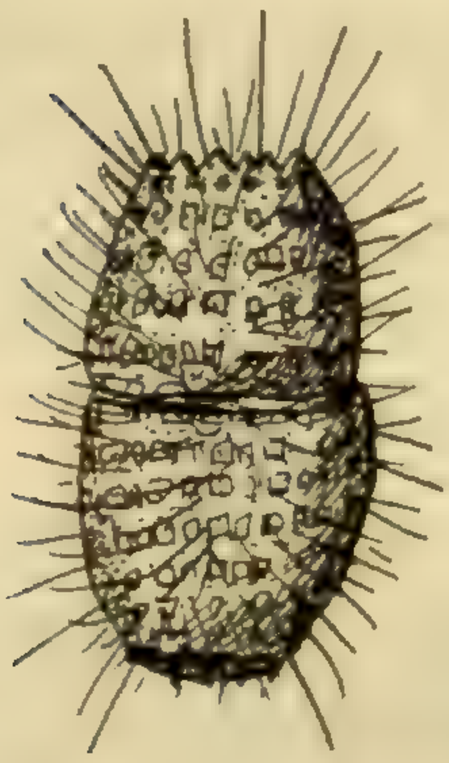


Fig.I Blepharisma lateritia

Fig.2 Synura uvella



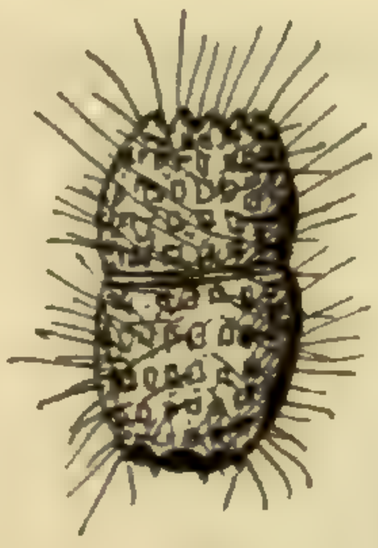


Fig.3 Colepa sp.

Fig.4 Colepa hirtus

Fig.5 Lacrymaria olor

Fig.6 Loxophyllum sp.

Fig.7 Astasia contorta

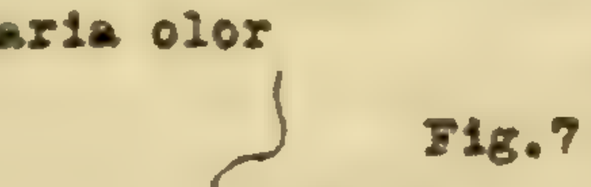
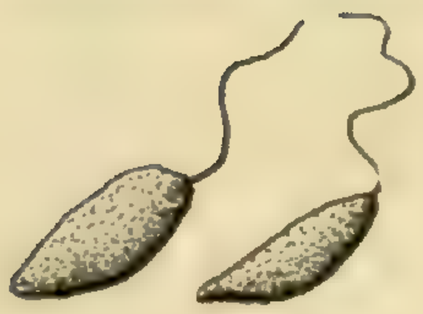


Fig.9 Cercomonas crassicauda

Fig.8 Urocentrum turbo

Fig.10 Cercomonas termo,normal and abnormal forms

PLATE II



Monas Dallingeri

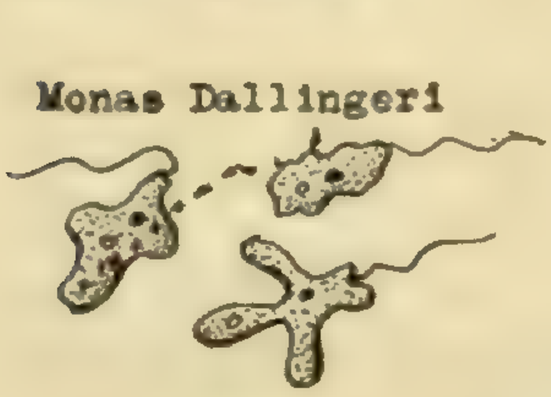


Fig.12 Mastigamoeba sp.

Monas fluida

Fig.11

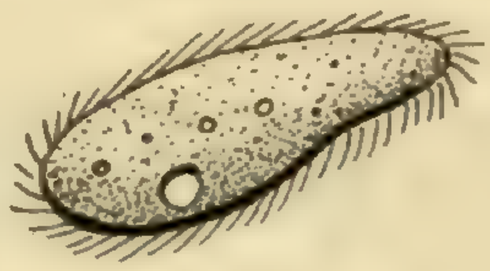


Fig.13 Enchelys sp.

Monas irregularis

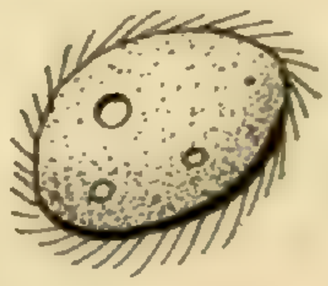


Fig.14 Holophrya sp.

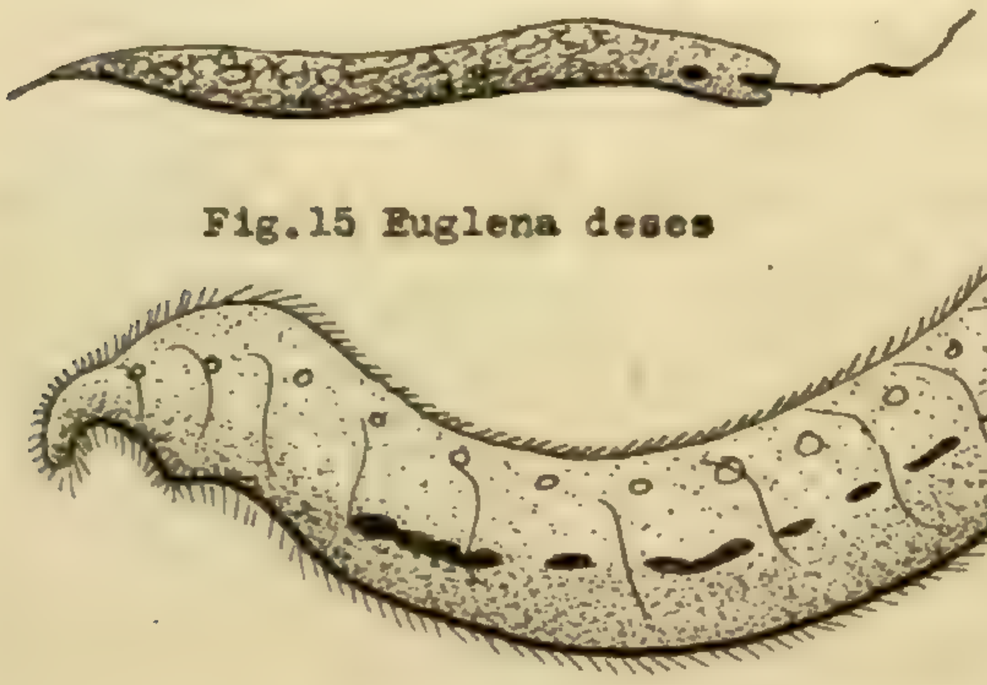
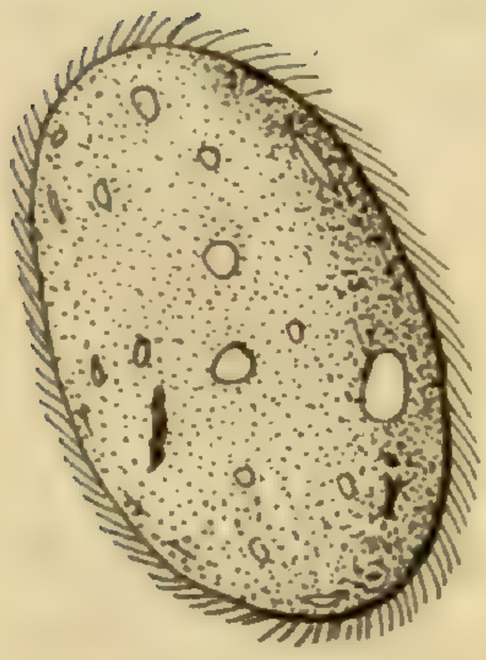


Fig.15 Euglena deses

Fig.20 Trepomonas agilis

Fig.16 Dileptus gigas



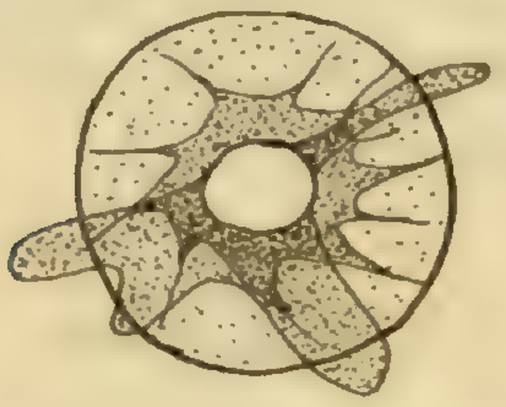


Fig.17 Arcella discoides

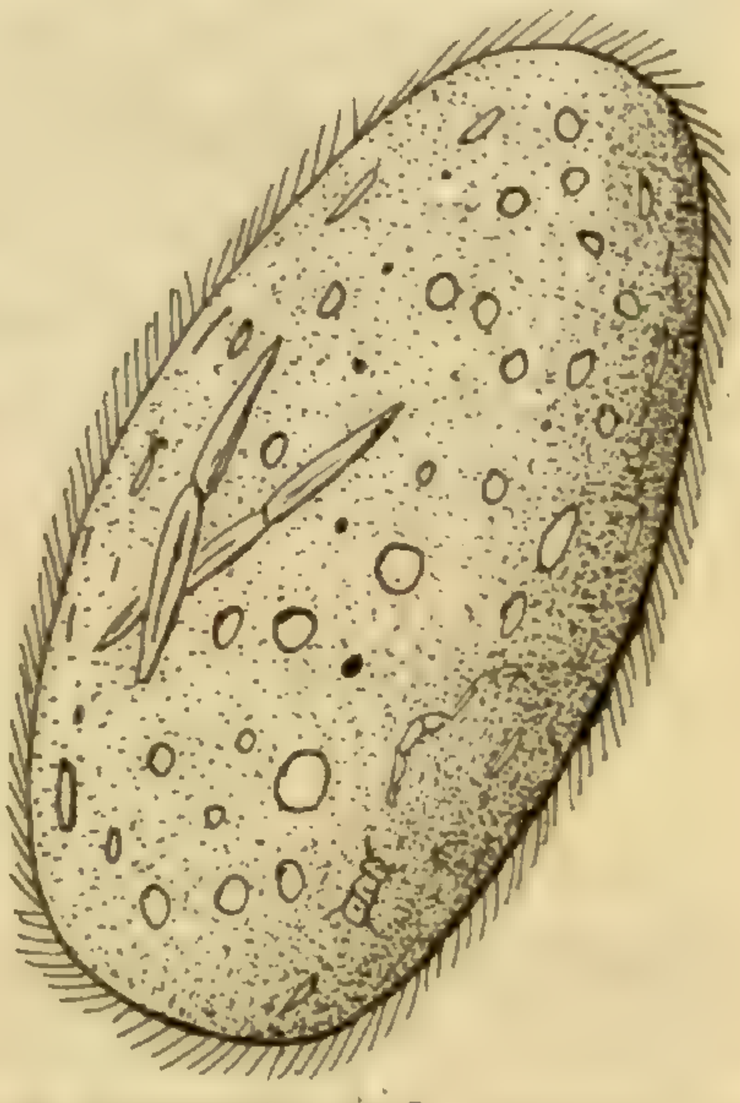


Fig.19 Frontonia sp.2

Fig.18 Frontonia sp.1

The body of *Loxophyllum* (Fig. 6) is surprisingly elastic. For this reason the creature undergoes rapid variations in length. Even within the confinement of a viscous gelatin solution which checks the motions of all of the other ciliates its strength is such as to enable it to perform various evolutions over the slide. In staining it frequently dies in a contorted and contracted condition. Before staining, it should be narcotized with a weak ethyl alcohol or killed with .01 per cent. osmic acid.

In *Astasia* (Fig. 7) the smaller secondary flagellum is sometimes either unusually filamentous, and therefore invisible, or lacking altogether. In many individuals no amount of manipulation of light directions and intensities or stains will reveal the smaller flagellum.

Urocentrum turbo (Fig. 8) can always be recognized by the frenzied and jerky rotation which accompanies its extremely rapid movement through the water. It usually comes to rest either close beside or buried among masses of algæ or other convenient material. In this resting condition it is liable to be overlooked. At such times its only evidence of activity is a scarcely perceptible twitching. This hiding in algal masses has all the appearance of being a deliberate attempt at concealment. Suddenly without warning its crazied rotation through the water is begun again. When swimming with retarded velocity through a gelatin solution, functional anterior and posterior portions of the body are recognizable.

ENVIRONMENTAL TYPE NO. II: CLEAR COLD WATERS LACKING PLANT GROWTHS

Sediment, composed of quartz and shale sands free from organic silt and supporting no plant life save a few Diatoms, in clear, pure, cold lakes and streams; temperature of water, cir. 56° F.

Predominant forms:

Astasia sp., abundant
Holophrya sp., numerous
Holostichia vernalis, numerous
Nostolenus orbicularis, several
Nostolenus sp., several

Other forms present in less numbers (few or rare):

Cercomonas termo
Cercomonas crassicauda
Chlamydomonas sp.
Colpidium sp.
Euplotes sp.
Lionotus sp.
Paramœcium bursaria
Stylonychia mytilus

The nucleus of *Cercomonas crassicauda* (Fig 9) is located near the functional anterior end of the body, and is not easily seen. The anterior flagellum is likewise difficult to distinguish, even upon staining, being exceedingly filamentous. It seems to have little to do with locomotion, and I believe that it is used principally as a sort of antenna. The propulsion of the creature is apparently accomplished by the lashings of the stout posterior flagellum, no matter which end of the organism is directed forward.

Cercomonas termo (Fig. 10), from 5 to 15 microns, is extremely variable in shape in the adult form. Its commonest and therefore characteristic appearance is roughly heart shaped, the flagellum arising from the broader extremity of the body. The young are not so variable, but although constant in general form, confusingly resemble the monads, in particular *Monas irregularis*. Their minuteness (.5 to 2 microns) makes them difficult to identify. They should be stained with a very weak solution of iodine or acetic methyl green, since the stronger stains, used for the larger Protozoa, cause them to lose their characteristic outline and to disintegrate in a short time.

Environmental Type No. III: Clear Flowing Water with Abundant Plant Life

Clear springs and streams supporting vigorous growths of such algæ as *Spirogyra, Drapernaldia,* etc., and water cresses; bottoms covered with Diatoms (often chiefly *Meridion circulare*), Desmids, and *Oscillatoria.* Temperature of water, cir. 66° F.

Predominant forms:

Amœba proteus, abundant
Amœba proteus, flagellospores (?), abundant
Chilodon cucullus, abundant
Chilomonas paramœcium, abundant
Colpidium sp., several
Colpoda inflata, several
Difflugia constricta, numerous

Difflugia globulosa, numerous
Difflugia lobostoma, numerous
Holophrya sp., several
Holostichia vernalis, several
Monas fluida, abundant
Monas irregularis, abundant
Monas Dallingeri (?), abundant
Nostolenus orbicularis, numerous
Oxytrichia pellionella, abundant
Prorodon teres, numerous

Other forms present in less numbers (few or rare):

Arcella artocrea
Aspidisca costata
Astasia contorta
Cercomonas crassicauda
Chilodon sp.
Chlamydomonas sp.
Colpidium striatum
Dileptus gigas
Enchelys sp.
Epiclintes radiosa
Euglena viridis
Euglena minima
Frontonia sp. 2
Holophrya sp.
Lionotus wrzesmosky
Mastigamœba sp.
Onychodromus grandis
Oxytrichia bifaria
Phacus sp.
Paramœcium caudatum
Stylonychia mytilus
Stylonychia pustulata

The identification of the *Monas* species (Fig. 11) is very difficult. Members of this genus resemble some of the members of the genus *Bodo*. Because of the hyaline character of the body its outline is not easy to distinguish. I have found that the most successful method of treatment before identification is attempted, is staining with a strong aqueous solution of iodine and potassium iodide. This both kills and stains. Examination must be made almost immediately since the organisms begin to lose their characteristic form in a short time.

Within the genus *Holophrya* have been provisionally placed a number of forms very closely allied in general characters. For a considerable number of these a separation into species has not yet been made.

The pseudopodia of the Mastigamœbæ (Fig. 12) do not invariably disappear with the appearance of the flagellum. They often remain, though much diminished in size, and even exhibit appreciable movement. Such movement, however, does not appear to aid in locomotion.

Enchelys (Fig. 13) is very like *Holophrya* (Fig. 14) and only under the most favorable conditions of staining and lighting can the side opening buccal orifice be definitely located. The proboscis-like projection at the anterior extremity of the body is subject to considerable modification, and can not be relied upon as a distinguish-

ing feature, but the position of the buccal cavity is virtually constant.

Euglena deses (Fig. 15) is recognizable by its enormously elongated body, for the propulsion of which its small flagellum seems inadequate. It frequently assumes an almost amœboid form, and not infrequently contracts to such an extent as to become nearly globular. Being variable in size, its form, when swimming by means of its flagellum, offers the readiest means of identification.

One particular locality, given especial notice, deserves detailed mention. This was a spring pool in a boggy upland meadow, almost hidden among large tufted clumps of luxuriant sedge-like grasses. The depth of the pool was about four inches, and its area about six square feet. Although exposed to the rays of the sun during the greater part of the day, yet the temperature of the water was kept low by a constant trickle of cold water from seepage springs in the glacial clays which underlay several acres of the region. The iron content of the water was unusually high, though the pool was crystal clear. Of higher aquatic plants there were none; Desmids were numerous; Diatoms were sufficient in numbers to form a brown film over the entire bottom. The pool contained seven large black-nosed dace, and other adjacent pools harbored several more. Snails, *Limnea* and *Physa* crawled everywhere. With the exception of half a dozen large *Hydrophilidæ* there were no aquatic insects, and but very few *Entomostraca.*

The predominant forms were *Monas fluida* and *irregularis*, occurring not alone in the bottom film but everywhere throughout the water and in great numbers. One species of *Holophrya* and *Cercomonas crassicauda* (but few individuals of each) completed the census of the forms.

Environmental Type No. IV: Clear Small Pools with Abundant Decomposing Organic Sediment

Small clear pools in depressions in rocks containing decaying leaf material and a small quantity of such algæ as

Spirogyra, Zygnemea, Mougeotia, etc., Desmids and Diatoms abundant. Water kept pure and cool by influx from small rills or by seepage from adjacent stream. Temperature of water, cir. 60° F.

Predominant forms:

Chilodon cucullus, numerous
Chilomonas paramœcium, abundant
Coleps hirtus, numerous
Difflugia globulosa, numerous
Holophrya sp., numerous
Holostichia vernalis, numerous
Monas sp., abundant
Paramœcium bursaria, numerous
Trachelocerca olor, numerous
Vorticella nutans, several

Other forms present in less numbers (few or rare):

Amœba proteus
Anisonema obliqua
Arcella vulgaris
Aspidisca costata
Chilodon vorax
Chlamydomonas sp.
Colpoda cucullus
Cyphoderia ampulla
Dallasia frontinia
Difflugia acuminata
Difflugia pyriformis
Dileptus gigas
Dileptus monilatus
Euglena deses
Euglena viridis
Euglypha alveolata
Euplotes sp.
Frontonia sp.
Hyalosphenia papilio
Loxophyllum sp.
Nostolenus orbicularis
Oxytrichia bifaria
Oxytrichia pellionella
Paramœcium caudatum
Pleuronema sp.
Prorodon armatus
Stylonychia pustulata
Stylonychia putrina

Dileptus gigas (Fig. 16) is surely the king of beasts among the ciliate Protozoa. It is entirely carnivorous and its appetite is apparently insatiable. The prey is stung by the well developed trichocysts which *Dileptus* bears upon its long "neck" and if too large to be swept into the buccal cavity by the cilia is forced in by the writhings of the "neck." The creature varies greatly in size, but is normally about 450 microns in length. Individuals have been reported measuring 800 microns!

ENVIRONMENTAL TYPE NO. V: POOLS CHOKED WITH ALGÆ; WATER WARM

Ditches and pools choked with heavy, luxuriant masses of algæ (*Spirogyra, Ulothrix, Zygnemea,* etc.) and exposed to the sun during the entire length of the day. *Cyclops, Canthrocamptus, Gammarus, Daphnia, Cypris, Simocephalus,* etc., abundant. In lesser numbers: aquatic

insects (especially the *Hydrophilidæ*), *Limnea, Physa, Planorbis,* etc. Temperature of water, cir. 70° F.

Predominant forms:

Cercomonas termo, abundant
Chlamydomonas sp., numerous
Difflugia globulosa, numerous
Euglena viridis, very abundant
Monas fluida, very abundant
Monas irregularis, very abundant
Peridinium cinctum, numerous
Synura uvella, numerous
Trepomonas agilis, very abundant

Other forms present in less numbers (few or rare):

Actinophrys sol
Arcella discoides (?).
Arcella mitrata
Arcella vulgaris
Colpoda campyla
Cyphoderia ampulla
Frontonia sp.
Vorticella nutans

The species which I identified as *Arcella discoides* (Fig. 17) was much smaller than usual. Although there is considerable variation in size among the members of *Arcella* and particularly among *Arcella discoides* and *vulgaris,* yet I have never seen recorded individuals so small. The average diameter of twenty-five individuals from one sample was 70 microns.

The *Frontonia* species (Figs. 18 and 19) are very fully equipped with trichocysts. When irritated with acetic acid and then stained with iodine or methyl green they show beautifully. For use in the laboratory in the demonstration of the trichocysts of the ciliata they offer the best possible material. The species are not very common, however, and I know of no culture medium.

Trepomonas agilis (Fig. 20) varies greatly in size. Its apparent variation in shape can be explained, I think, by the fact that it swims sometimes with one aspect of the body presented to the observer and sometimes with another. The curious irregularity of its body would therefore allow it to show a number of different outlines. Often it swims in one position for a long period, and again it twirls rapidly about going through the whole gamut of its apparent changes of form in a few seconds.

In one typical roadside ditch overhung with grasses and weeds and literally filled to overflowing with rich masses of *Spirogyra* I found an almost pure culture of *Euglena*

viridis. The only other forms present were but very few *Colpoda campyla* and *Cyphoderia ampulla*.

Several samples were taken from leaves and grass frozen together, lying beneath three inches of solidly compacted snow in a small oak grove not far from a ravine through which flowed a perennial stream. This material was allowed to stand in its own snow in a cotton-tamponed jar until the snow had melted and the resulting water had attained room temperature, cir. 72° F.

The *Amœba proteus* were more abundant than I had ever seen them before except in artificial cultures. Not infrequently as many as ten individuals could be counted at once in the field of the 10 mm. objective. The other predominant forms were:

Holostichia vernalis, abundant.	***Opalina ranarum***, several
Monas irregularis, abundant.	***Paramœcium caudatum***, several

Other forms present in less numbers (few or rare):

Astasia lagenula	***Paramœcium trichium***
Oikomonas sp.	***Platyrichotus opisthobolus***

CATALOGUE OF SPECIES NOTED WITH MEASUREMENTS IN MICRONS

In each case *length* refers to the antero-posterior axis, except with globular or subglobular forms when it refers to the diameter of the body. All dimensions are given in microns (=1/1000 mm.).

It is interesting to recall, in connection with the sizes of these organisms, that the diameter of the average human hair is 100 microns.

Genus	Species	Length
Actinophrys	***sol***	60
Amœba	***limax***	50–60
Amœba	***proteus***	125 (variable)
Amœba	***radiosa***	50–100 (variable)
Anisonema	***obliqua***	
Arcella	***artocrea***	160
Arcella	***dentata***	95
Arcella	***discoides***	115
Arcella	***mitrata***	100–150
Arcella	***vulgaris***	55 (variable)
Aspidisca	***costata***	30

Astasia	*contorta*	30 (variable)
Astasia	*lagenula*	30
Astasia	sp.	75
Biomyxa	*vagans*	50 (variable)
Blepharisma	*lateritia*	170
Carchesium	*polypinum*	60 (bell only)
Centropyxis	*aculeata*	125–150
Cercomonas	*crassicauda*	25
Cercomonas	*termo*	5–15
Chilodon	*cucullus*	135
Chilodon	*vorax*	70
Chilodon	sp.	42
Chilomonas	*paramœcium*	40 (variable)
Chlamydomonas	sp.	17
Codonocladium	*umbellatum*	8–12
Coleps	*hirtus*	50
Coleps	sp.	30
Colpidium	*striatum*	45
Colpidium	sp.	34
Colpoda	*campyla*	70
Colpoda	*cucullus*	95
Colpoda	*inflata*	45
Colpoda	sp.	50
Cothurnia	*maratima*	85
Cyphoderia	*ampulla*	75
Dactylosphærium	*radiosum*	25 (variable, body only)
Dallasia	*frontinia*	150
Difflugia	*acuminata*	100–300
Difflugia	*constricta*	50–100
Difflugia	*corona*	150–250
Difflugia	*globulosa*	20–50
Difflugia	*lobostoma*	90–120
Difflugia	*pyriformis*	250–350
Dileptus	*gigas*	450 (variable)
Dileptus	*monilatus*	200
Dinomonas	*vorax*	18 (variable)
Enchelys	sp.	50
Epiclintes	*radiosa*	50
Euglena	*deses*	50–200
Euglena	*minima*	30 (variable)
Euglena	*viridis*	50 (variable)
Euglypha	*alveolata*	75
Euplotes	*carinata*	80
Euplotes	*charon*	90
Euplotes	sp.	35
Frontonia	sp. 1	300–450
Frontonia	sp. 2	100
Halteria	*grandinella*	25
Heterophrys	*myriapoda*	60
Holophrya	sp.	25–45

Holostichia	*vernalis*	50
Hyalosphenia	*papilio*	125
Lacrymaria	*olor*	
	extended	360
	contracted	90
Lionotus	*wrzesmosky*	225
Lionotus	sp.	30
Loxophyllum	sp.	50
Mastigamœba	sp. (*repetans?*)	20
Monas	*Dallingeri*	These vary between 1 and 5
Monas	*fluida*	
Monas	*irregularis*	
Nostolenus	*orbicularis*	35
Nostolenus	sp.	17
Oikomonas	sp.	10–20
Onychodromus	*grandis*	100–350
Opalina	*ranarum*	115
Oxytrichia	*bifaria*	100–280
Oxytrichia	*pellionella*	80–150
Paramœcium	*bursaria*	90–150
Paramœcium	*caudatum*	150–300
Paramœcium	*trichium*	85
Phacus	sp.	80
Platyrichotus	*opisthobolus*	50
Pleuronema	sp.	50
Prorodon	*armatus*	25
Prorodon	*griseus*	40
Prorodon	*teres*	150
Stentor	*cæruleus*	100–400
Stentor	*polymorphus*	100–300
Stylonychia	*mytilus*	80–150
Stylonychia	*pustulata*	80–150
Stylonychia	*putrina*	50 (variable)
Synura	*uvella*	
	individual	10
	colony	35
Trachelocerca	*olor*	70–130
Trepomonas	*agilis*	8–10
Trichodina	*pediculus*	230
Trinema	*acinus*	60
Urocentrum	*turbo*	45
Volvox	*globator*	-
Vorticella	*microstoma*	45
Vorticella	*nutans*	70
Vorticella	sp.	60

THE RÔLE OF ISOLATION IN THE FORMATION OF A NARROWLY LOCALIZED RACE OF DEER-MICE (PEROMYSCUS).*

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No one who has critically examined large numbers of specimens, belonging to such a widely distributed and diversified genus as *Peromyscus*, can fail to be impressed with two facts. First, the differences upon which the so-called "subspecies" are based are real and obvious ones. But, secondly, the actual subspecies which are recognized and named are necessarily highly artificial groups. On the one hand, each subspecies intergrades with others to such an extent that the assignment of a given specimen to one or the other group is often quite arbitrary. And on the other hand, even these "subspecies" themselves are far from being elementary. They are composite groups, comprising, in many cases, a number—perhaps a great number—of distinguishable local types. The word *distinguishable* is here used in a qualified sense. It is likely that the distinctions would commonly be obvious just in proportion as the collections were made at points which were remote from one another.

Indeed, it has been said by one who has monographed this genus of mice[1] that "classification becomes . . . like dividing the spectrum and depends largely upon the standards set, for, theoretically at least, the possibilities of subdivision are unlimited (p. 17)."

None the less, it is generally believed that where well-marked physical or other barriers are interposed between two groups of individuals, this continuous intergradation

*Read before Ecological Society of America, San Diego meeting, August, 1916.

[1] Osgood, "Revision of the Mice of the American Genus, *Peromyscus*," North American Fauna, No. 28. Washington, 1909.

of racial characters may be largely interrupted. It is the object of this paper to discuss a case of this sort which I have had the opportunity of studying during the past year.

The subspecies *Peromyscus maniculatus rubidus*, according to Osgood,[2] who first described it, occupies a strip of varying width on the "coast of California and Oregon from San Francisco Bay to the mouth of the Columbia River." In discussing certain local variations shown by this subspecies throughout its range, the same writer states that "six specimens from the Outer Peninsula, near Samoa, Humboldt Bay, are decidedly paler than others from the neighboring redwoods. They evidently represent an incipient and very local subspecies, and well illustrate the plasticity of the group to which they belong." Osgood further remarks that "a careful study of this variation and the local conditions doubtless would prove instructive" (p. 66).

During the latter part of May, 1916, I trapped on two consecutive nights in the neighborhood from which Osgood obtained his six "aberrant" specimens of *rudibus*.[3] About one hundred live-traps were set on each occasion. Twenty-eight specimens were taken, of which twenty-one were later available for skinning and for careful measurement. These last were all in either mature or adolescent pelage, and were about evenly divided in respect to sex.

The distinctness of this race from the *rubidus* of the redwood forests on the mainland was evident from a casual inspection of the living mice. A more careful comparison of freshly killed specimens from the two localities, and later of their prepared skins, justifies the following generalizations. These impressions were formed independently by several other persons to whom I showed the specimens, and were confirmed by more careful ex-

[2] *Loc. cit.*, p. 65.

[3] The trapping was done between one and two miles northwest of the village of Samoa. Besides these *Peromyscus*, the only other animal caught was a single specimen of *Microtus*.

amination and measurement. (1) The Samoa lot, as a whole, were paler than the redwood lot; (2) the tails of the former were shorter, and (3) the ears were longer.

To consider first the coat color, the mean difference between the two series of skins is evident at a glance. Likewise, it is plain that the palest Samoa specimen is paler than the palest Eureka (redwood) specimen, and that the darkest among the former is paler than the darkest among the latter. It must be admitted, however, that the two series overlap rather broadly,[4] the darker skins of the Samoa stock being as dark as or darker than the paler ones of the Eureka stock.

An attempt to express the color of a mammal's pelage in terms of any set of "standard" colors is beset with great difficulties. Instead of a uniformly tinted, plane surface, we have to do with a mixture of variously colored hairs, further diversified by minute shadows and reflections. I have, nevertheless, endeavored, in a rough way, to "match" the colors of these two races with those of Ridgeway's "Color Standards and Color Nomenclature."[5] In the Samoa race, the general tone of the lateral regions of the body lies between the "tawny olive" and "Saccardo's umber," that of the dorsal darker stripe being not far from "sepia." In the Eureka mice, the lateral regions range from "Saccardo's umber" to "sepia," the dorsal stripe being of a depth somewhere between "sepia" and black. These comparisons will at least enable the reader to judge of the degree of difference between the two racs.[6]

As regards the tail, it was plain without measurement that the average length of this member was greater in the

[4] I have at present for reference twenty-one skins of the Samoa lot and thirty skins of wild adults from the redwoods. Ten of the latter individuals were trapped and skinned at about the same time as the former, so that the factor of season may be disregarded.

[5] Washington, 1912. Published by the author.

[6] In my further studies of *Peromyscus* I plan to employ two revolving color-wheels, on one of which the skin itself will be rotated, on the other sectors of black, white and various primary colors. This apparatus is now being tested by Mr. H. H. Collins and myself.

Eureka than in the Samoa race, though here again the difference related to averages and did not hold for all individual cases.

A comparison of the mean figures for *absolute* tail length in two series of mice is not entirely justifiable, particularly if the two lots of individuals differ somewhat in mean body size. But the *relative* tail lengths (expressed as percentages of body-length) may be fairly compared, since there is good evidence that these ratios remain nearly constant after the first few months of life. The following table allows of a comparison between the two races, in respect to this character:

	Number of Cases	Mean (Percentage)	Standard Deviation
Eureka (males)	83	104.39 ± 0.37	4.95
Eureka (females)	53	103.60 ± 0.54	5.85
Samoa (sexes combined)	21	97.48 ± 0.94	6.38

The differences between the Samoa lot (sexes combined) and the Eureka males and females are 6.91 per cent. and 6.12 per cent., respectively. These differences are about seven and six times their probable errors, respectively. Their significance may therefore be regarded as fairly certain, despite the small numbers comprised in the Samoa series.

As regards foot-length, the two races do not differ significantly. But the ear, as already stated, is appreciably longer in the Samoa mice, this difference being perceptible, even without measurement. Here, as in the case of tail-length, a simple comparison of gross averages for the two groups would be unjustifiable. But in the present instance, the conversion of the absolute values into percentages of body-length would be equally unjustifiable, since the growth of the ear is not at all proportionate to that of the body as a whole. We must therefore resort to the method of "size groups," *i. e.*, we must divide each of our two lots of animals into small groups comprising individuals of nearly equal size.

In the case at hand, we have fifteen groups, or rather

pairs of groups, within which a comparison of average ear-length is possible. In twelve cases the mean figure is greater for the Samoa mice, in two cases it is greater for the Eureka mice, while in one case the two figures do not differ appreciably. The probabilities against such a preponderance being due to chance are of course high. The mean difference in ear-length between the two lots, computed according to a method described by me in an earlier paper,[7] is 0.87 mm. Those who have made careful measurements of mice will regard such a difference in the length of this appendage as far from trivial.

Let me now say something as to the environmental conditions under which these two races of *rubidus* live. Those which I have designated as the "Eureka" or "redwood" race were trapped by me during two different years, within a distance of two miles from the southern limits of the city of Eureka, California. The region is one covered in large part by redwood forest, most of which is of second growth, although there are some small areas that have never been logged. The predominant tree is the redwood (*Sequoia sempervirens*), but several other conifers are common, the most abundant of these being the Sitka spruce (*Picea sitchensis*), Douglas fir (*Pseudotsuga taxifolia*), and lowland fir (*Abies grandis*). The red alder (*Alnus rubra*), cascara (*Rhamnus purshiana*), waxberry (*Myrica californica*), red elderberry (*Sambucus racemosa*), and a willow (*Salix hookeriana*) appear to be the chief non-coniferous trees of this district.[8] The "wild lilac" (*Ceanothus thyrsiflorus*) is likewise common in some of the more open areas, often reaching the proportions of a small tree.

Except in recently cleared tracts, the region is one of dense underbrush, the shrubbery and vines forming, in fact, a veritable jungle which is frequently hard to pene-

[7] *Journal of Experimental Zoology*, Vol. 18, April, 1915, particularly, pp. 341 et seq.

[8] For the determination of many of the plants referred to in this paper I am indebted to Professor H. M. Hall, of the University of California, and to Mr. J. P. Tracy, of Eureka.

trate. Here we meet with the thimble-berry (*Rubus parviflorus* var. *velutinus*), the salmon-berry (*Rubus spectabilis* var. *menziesii*), huckleberry (*Vaccinium ovatum*), red bilberry (*V. parvifolium*), salal (*Gaultheria shallon*), and in the more open areas the blackberry (*Rubus vitifolia*). Two ferns (*Aspidium munitum* and *Pteris aquilina*) are extremely abundant, the latter in particular forming dense growths higher than a man's head. In the

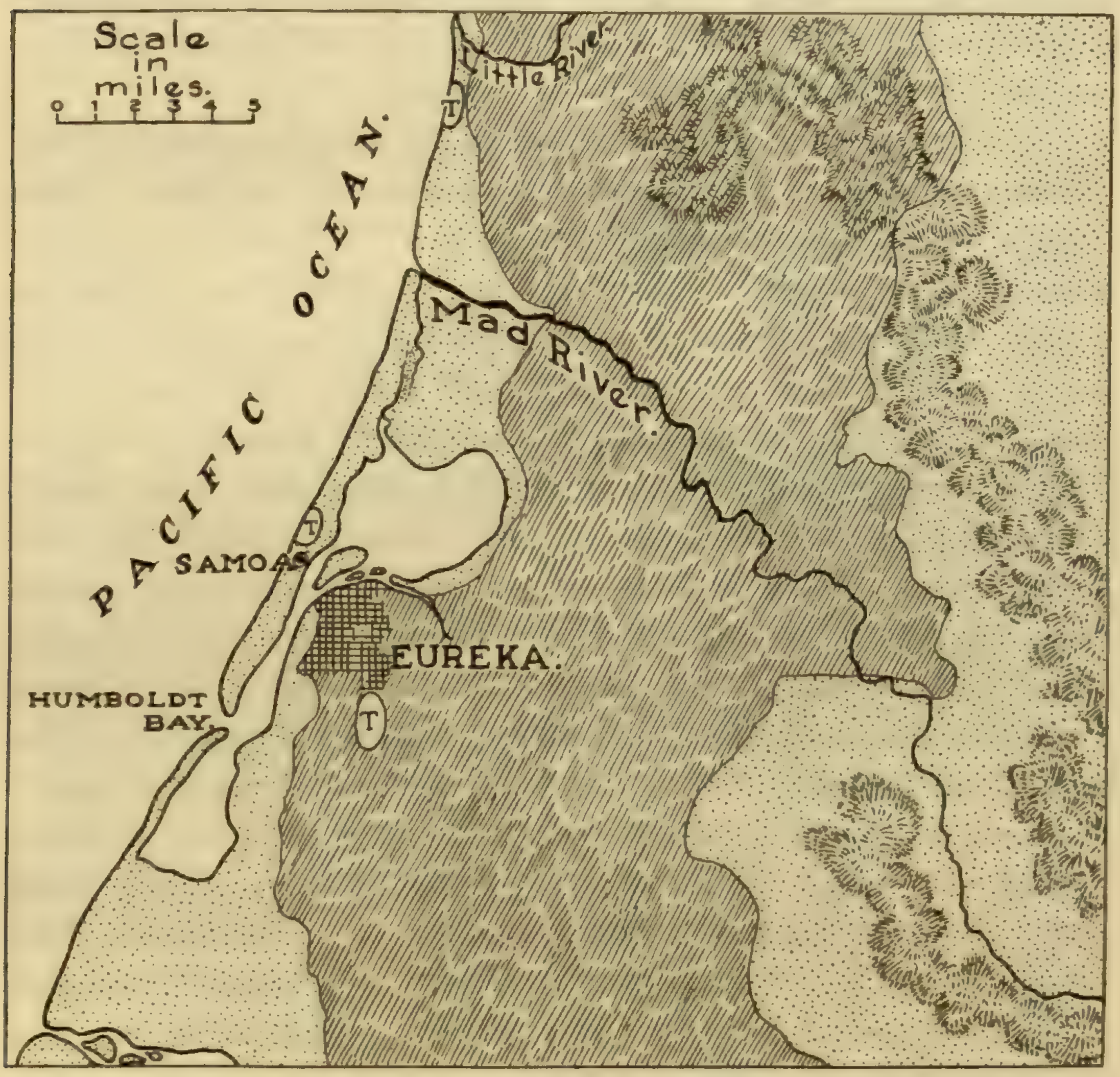

FIG. 1. Map of the vicinity of Humboldt Bay, California, based upon J. N. Lentell's map of Humboldt County. The three principal trapping stations are designated by the letter T. Area occupied by redwood forests is indicated by oblique shading.

more open areas a tall annual of the evening primrose family (*Epilobium angustifolium*) constitutes an important element in the vegetation.

One coming from the more arid parts of California can

not fail to be impressed by the prevailing humidity of both soil and atmosphere in this region. In the dense shade of the great redwoods the ground is damp, even during the summer months, and the fallen logs are covered with mosses and fungi.

When we cross Humboldt Bay to the narrow peninsulas separating this body of water from the ocean (Fig. 1), we enter a quite different environment. No redwoods are found, the woods, where present, are open, and the ground is prevailingly dry and sandy. In the wooded area, extending down the axis of the northern peninsula, the predominant tree is a small pine (*Pinus contorta*), though the waxberry and willow (*Salix hookeriana*) are likewise abundant, and small specimens of the Sitka spruce are fairly common. Among the more frequent shrubs are the huckleberry (*V. ovatum*), the twinberry (*Lonicera involucrata*) and silk tassel bush (*Garrya elliptica*). The ground is largely covered by two plants of trailing habit, the bearberry (*Arctostaphylos uva-ursi*) and the beach strawberry (*Fragaria chilensis*).

On its ocean side, the peninsula is bordered by a wide strip of shifting sand. Here the process of dune formation may be witnessed to perfection, the dunes often reaching a height of forty or fifty feet. In places the encroachments of the sand upon the hard-pressed vegetation are evidently rapid, solid ramparts of willows and spruces being steadily engulfed by an advancing wall, frequently as high as the trees themselves. Nevertheless, even on the open sands of the dunes, certain trailing plants maintain a precarious foothold. Among the commonest of these are to be mentioned the yellow sand verbena (*Abronia latifolia*), the beach strawberry (*F. chilensis*), beach pea (*Lathyrus littoralis*), and two species of Franseria (*F. chamissonis* and *F. bipinnatifida*), while the succulent *Mesembryanthemum aequilaterale* is occasionally met with.

Despite the nearness to the ocean and the high atmospheric humidity, the peninsula region seems dry in comparison with the redwood forests. This is due in part to

the loose, sandy character of the soil—where, indeed, any real soil exists—and to the comparative lack of shelter from the prevailing westerly winds. Evaporation here is doubtless more rapid than in the comparatively stagnant air of the forests.

To my surprise, the footprints of mice and other small mammals were abundant, even on the shifting sands, in the areas of sparsest vegetation. Since these tracks, for the most part, were effaced every day by the wind, the animals must have been present in large numbers. Indeed, it was in or close to the dune region that I trapped most of the twenty-eight *Peromyscus*. It seems more than possible, therefore, that the predominantly paler shade of the mice dwelling here may be due to the same causes which are operative in producing the yet paler hues of many of the desert rodents.

What the effective factors are can not yet be stated with certainty in either case. Protective coloration is of course an obvious explanation, but it is one of doubtful applicability in the case of animals which are almost wholly nocturnal in their habits. For this and other reasons it seems more likely that the pale coloration of these mice stands in some more direct relation to the humidity of their immediate surroundings. That it is not, however, a strictly "somatic" phenomenon, called forth anew in each generation, I have already shown for the desert race, *P. m. sonoriensis*.[9]

Whether or not the peculiar color of the pelage in the Samoa race is likewise hereditary I have endeavored to test experimentally. Seven living females and a number of males were brought to La Jolla in June, 1916. Unfortunately, it was not possible to obtain more than two broods of young, comprising three individuals, one male and two females. These animals were carefully examined at the age of five months, in comparison with over forty individuals, derived from the redwood stock, which were

[9] AMERICAN NATURALIST, Nov., 1915. I have since reared this race in Berkeley as far as the third (in one instance the fourth) cage-born generation, without any certain modification in color.

mainly of the same age or older, and likewise reared from birth at La Jolla. Not a single individual of the latter stock was as pale as either of the two females of Samoa parentage. The male of the Samoa race was, however, of about the average shade of the redwood descendants. As stated above, some of the wild parents, trapped on the peninsula, were likewise as dark as many of the redwood series.

No certain conclusions can, of course, be based upon these three individuals. But the condition of the two females certainly lends support to the belief that the peculiar coat color of the Samoa race, however it was acquired, has become fixed germinally.

Reference to the map shows that the northern peninsula of Humboldt Bay is largely isolated, so far as land-living rodents are concerned. In addition to the ocean and the bay, a marshy tract extends from the latter to the Mad River, which, in turn, interposes a further barrier on the north, and nearly converts the peninsula into an island. Beyond the mouth of Mad River, this same type of sand-dune formation extends uninterruptedly to the mouth of Little River, about six miles to the north, where it ends abruptly and the shore line becomes precipitous.

Now this northward extension of the sand-dune region is not isolated by any physical barrier from the redwood forest, which here comes near to the coast. It occurred to me, therefore, to attempt the collection of *Peromyscus* from a point somewhere within this region. The locality chosen was close to the ocean, about two miles south of Little River and four to five miles north of Mad River. Here the conditions were found to be closely similar to those on the exposed side of the northern peninsula of Humboldt Bay. The dunes were on the whole lower, however, and some minor differences were noted in the flora. The belt of shifting sand here ranges from five or six hundred feet to perhaps a fourth of a mile, giving place on the landward side to a narrow meadow or marshy area, succeeded by a high, steep, wooded ridge.

About ninety traps were set on two consecutive nights,

yielding in all forty-eight *Peromyscus*, all belonging to the subspecies *rubidus*. Many of these were still in juvenile pelage and such individuals were kept and allowed to mature in captivity.

A hasty comparison of the living Little River animals (as I shall call them) with those from the Samoa and Eureka trapping grounds made it plain that, in respect to color, they belonged with the latter group rather than the former. Careful comparisons of series of dead mice and of skins were made later and the bodies were subjected to the customary measurements. Owing to numerous deaths, however, only twenty-eight individuals were available for these purposes.

This more critical examination confirmed my earlier belief that the Little River mice agreed pretty closely, in average color, with the redwood stock, but that they differed widely from those taken on the peninsula. It seemed probable, however, that the mean shade was slightly lighter than that of the former animals, making them, to this extent, intermediate.

One conclusion then seemed plain. The peninsula race, exposed to certain modifying conditions, was enabled to differentiate from the mainland stock, owing to the almost insuperable barriers to migration. The Little River stock, exposed to practically the same conditions, have not formed a distinguishable race, because the rate of differentiation has been far exceeded by the rate of diffusion, or intermingling with the great body of more typical "*rubidus*," dwelling in the redwood forests which extend back from the coast. We might seem to have, therefore, a particularly clear cut example of the effectiveness of isolation in the formation of a local race.

Now, I am not yet prepared to admit that such conclusions would be groundless. But here, as so often happens, a further study of the data has shown that the problem is more complex than was at first suspected. It is true that the mice of the more northern sand dunes have not formed a distinct race as regards *color*. But it is none the less certain that they differ from those of the

Eureka region in regard to both the length of the tail and that of the ear. In respect to the former character, they agree pretty closely with the Samoa race, the difference from the redwood stock being statistically even more certain in this case. To still further complicate the situation, we find that the ear, instead of being longer, is shorter than that of the redwood mice by about half a millimeter, and thus averages about one and one half millimeters shorter than in the peninsula race. Here, too, the differences are even more certain statistically than those which distinguish the Eureka and Samoa series.

The numbers are small, of course, only twenty-eight of the Little River mice having been available for measurement. But as regards tail length, the difference between the averages is seven to nine[10] times its probable error, so that the likelihood of its being due to random sampling is very small.

Have we, then, here merely another example of inconclusive data, which might best have been left unpublished? I do not think so. The mere existence of these local differences in color and in the size of parts deserves careful description, whatever interpretation we may place upon them.

Moreover, I am disposed to believe that the case of coat color is not entirely comparable with that of the length of the appendages. In another article[11] I have given reasons for thinking that some of the differences in the former may have arisen in nature as more or less direct effects of environmental conditions. On the other hand, I have shown that such an explanation would be of very difficult application as regards some of the measurable differences in the parts of the body, even though the latter are known to be readily influenced by various experimental agencies.

Now the evidence at hand is sufficient to show that any environmentally produced modifications of coat color are

[10] Depending on whether the comparison is made with the Eureka males or females, the sexes being combined in the case of the Little River group.

[11] AMERICAN NATURALIST, Nov., 1915.

at best rather gradual. *Rubidus* remains *rubidus* and *sonoriensis* remains *sonoriensis*, after several generations of captivity in changed climates. But even the first cage-born generation of each of my subspecies is found to be highly modified by confinement, in respect to the mean length of certain of the appendages. That this somatic plasticity would be accompanied by a high degree of germinal instability, as regards these parts, could not, of course, be predicted in advance. But the frequent appearance of local differences of type renders it probable that this is true. Whether or not these local peculiarities are due in some indirect way to environmental factors, or whether they are due to "spontaneous" mutation, need not concern us here. The main point to bear in mind is the probability that the pelage color is somewhat more stable in these mice than are the bodily proportions, despite the fact that it is the former, rather than the latter, which gives the clearest evidence of a definite correlation with known factors of the environment.

For the reason just stated, it is possible that the differentiation of a new color race might require fairly rigid isolation; whereas local differences in some of the measurable parts might arise in the presence of no other barrier than the naturally slow rate of diffusion of a non-migratory animal. As was remarked earlier, we have reason to suppose that representative collections from an indefinite number of localities would reveal the existence of statistically certain differences between the mice of many of these localities. In most cases, it would probably be unjustifiable to assign these series to distinct *races*, or other definite taxonomic groups, since it is likely that perfect intergradation would be found between most of them, and that the degree of difference would be largely a function of the distance apart of their respective habitats.

These last remarks are, of course, largely conjectural. Part of the author's present program consists in a careful study of local differences of the sort here discussed.

It is hoped that this will render possible more definite answers to some of these difficult questions.

It seems to be held by certain zoologists that any discernible difference between two local types, if at all constant, ought to be in some way recognized in the nomenclature. Indeed, I have been advised to name this modified race of *rubidus* from the northern peninsula of Humboldt Bay. Such a practise, if carried out consistently, would lead either to an endless multiplication of subspecies, or else to the introduction of quadrinomial names. Either procedure would, I think, be deplorable. The actual needs of the situation can commonly be met, I believe, by stating the locality from which a given specimen or collection was taken. The bestowing of formal names creates the false impression of a multitude of well-defined entities which do not, in reality, exist. Moreover, it is my firm conviction that nomenclature should have for its object the recognition of resemblances as well as the recognition of differences. The first of these functions is all too frequently overlooked.

SHORTER ARTICLES AND DISCUSSION

THE MIGRATION OF FISHES

Under the head of "The Migrations of Fish,"[1] Professor Alexander Meek has given a voluminous account of what is known of the movements and the distribution of the various families of fishes. The work is illustrated with drawings and photographs of many species, showing not only their forms and their movements, but often the stages of development and the structure of fins and scales. Especially valuable is a series of maps showing the geographical distribution of interesting groups. The word migration is taken in its largest sense, including not merely movements of individuals or of masses, but the larger problems of distribution, extending often over geological periods.

It is plain that distribution is intimately related to migration and migration to currents.

As the problems of fish conservation depend directly on the facts of migration and distribution, especial attention is given to the development and movements of food fishes and naturally to those of the North Atlantic.

After a general discussion of the continental and oceanic changes which have taken place since Eocene times, these having a direct bearing on modern conditions of fish-distribution, Professor Meek takes up the various groups of fishes, beginning with the lowest, treating of the habits, movements and distribution of each group in turn.

The excellent account of the lampreys and hag-fishes shows a certain omission. While the lampreys fasten themselves to other river fishes, sturgeons, catfishes and the like, rasping great holes with their teeth, the hag-fishes attack the throats of large sea-fishes, entering the muscular system and almost destroying it before the fish concerned finally dies. Around Monterey Bay, various flounders and rock-fishes (*Sebastichthys*) are thus attacked and drift about as living hulks while the hag-fish (*Polistotrema*) devours their muscular tissues.

The interesting parallelism in habits and distribution of the

[1] "The Migrations of Fish," by Alexander Meek, M.Sc., professor of zoology, Armstrong College in the University of Durham, and director of the Dove Marine Laboratory, Cullercoats. Edward Arnold, London, Longmans, Green & Co., New York. Price $4.50.

sturgeon and the lamprey is noted by Professor Meek and in both cases the facts now observed are of long standing. Both had apparently "refuge-regions" during glacial times. We do not, however, see the reason for the suggestion that the green sturgeon of California (now almost extinct) "may have been derived from the Atlantic during the post-glacial disturbance."

The migrations and breeding habits of the herring are treated with special fullness, proportionate to the economic value of the species. For in the north, as Björnson informs us, wherever a herring school touches the coast a town springs up, like driftwood on the beach.

In the account of the trout, very good as a whole, we may note that the genus *Salmo* is represented by different forms, originally derived from the Pacific coast, in the Great Basin of Utah, and also in the headwaters of the Colorado, Rio Grande, Arkansas and Platte, as well as the Columbia and Missouri. It is probable that the freshwater *irideus* (Rainbow trout) and the sea-run *gairdneri* (steelhead) are not really different, but both are quite separate from the cutthroat trout (*Salmo clarki*, wrongly identified at first by American authors with the Kamchatkan *Salmo mykiss*) from the Tahoe trout (*Salmo henshawi*) and from the several local forms which have sprung from these or which have preceded their advent. The last seems to be the case with the silver trout of Lake Tahoe (*Salmo regalis*). The suggestion of Professor Meek that the European salmon (*Salmo salar*) of the Miocene was divided into a North Atlantic and a Mediterranean form is interesting. The latter developed as a "trout" dividing into sea trout (*Salmo trutta*) and burn (or brook) trout (*Salmo fario*). But these two are as yet not really differentiated, corresponding in a way to the rainbows and steelheads of the Pacific coast. Professor Meek says:

> There is thus good reason for believing that the sea trout and the common trout may be the same, the one retaining the migratory habit and the other confining itself to fresh water.

Our own experience with the species lends probability to this view.

That "the salmon preceded the trouts" in time is also probable, but the western species of trout must have been derived from the trout of Europe and Asia.

The Pacific salmon must be older and more primitive than the Atlantic salmon, for the six species differ from one another, more than any trout or even the Atlantic salmon itself differs from any other black-spotted trout whatever.

Professor Meek hardly does justice to the spawning habits of the red or blueback salmon. It runs up rivers to varying distances—from one mile to 1,500 miles (Lake Labarge on the Yukon). But it never enters a stream which does not flow from a lake and it spawns always in the small streams at the head of the lake.

At Boca de Quadra in Alaska, the small stream is barely a mile long. It comes from a clear lake, perhaps five miles long. Into this stream and lake the salmon crowd by the thousands. The Yukon is not a good red salmon stream, because the nearest tributary lake, Labarge, is about 1,500 miles from the sea. Yet red salmon enter the river and reach the lake. In streams without lakes as the Skagway, red salmon are never seen. The King salmon (*Oncorhynchus tschawytscha*) also runs for great distances, but it is absolutely indifferent to the presence of lakes. It is probable that the red salmon spend their first winter in the lake and some never leave it, remain landlocked and dwarf until spawning time (usually four years).

One of the most difficult of problems is to understand the instinct of the red salmon. Every individual of this and of each of the other species of Pacific salmon (*Oncorhynchus*) dies after spawning. How does the spawning fish, stupid in most regards, know when it enters a river that there is a lake before it? How does it come to avoid all lakeless tributaries as it goes up, finally reaching the lake's head and the brooks that feed it? And why do the other salmon species totally lack this instinct? There are other problems, yet unsettled, regarding the supposed homing instincts of salmon. The majority (but not all) seem to return to spawn to the parent stream which they left as fingerlings. Why not all? And why any?

The recognition of the age of salmon and trout by the adjustment of the rings on the scales, as recently worked out by Dr. C. H. Gilbert and others, received full attention from Professor Meek. The scales of the salmon are marked by concentric rings of growth, and these are more widely separated in the summer, the feeding time of the salmon when the individual grows most rapidly.

Professor Meek devotes much space to the singular breeding habits of the eel, which spawns in the sea, entering rivers to feed. But many individuals, in our Mississippi Valley never descend to the sea. A very large eel, once taken by the present reviewer, above the Cumberland Falls in Kentucky, 2,000 miles from the

sea, must either have never spawned or cast its spawn into the river.

The larvæ of eels as well as of some other soft-rayed species are quite pellucid, without pigment cells and with "a roomy space between the skin and the muscles, distended by a watery fluid." Many of these larvæ, in their transformation to the condition of young fishes become much reduced in size, though increasing in weight, by the obliteration of these interspaces.

Professor Meek's studies pass through the whole long series of fish-families. For want of space, we may not follow them further in these pages. We must give the work, as a whole, very high praise as carefully, intelligently and scientifically done, and as constituting a reference book of great value. The author has well covered the range of the periodicals which treat of the distribution and habits of fishes. He seems, however, to have overlooked the most extensive recent work of a similar range, "Jordan's Guide to the Study of Fishes," published in New York in 1905.

DAVID STARR JORDAN

NEW LIGHT ON BLENDING AND MENDELIAN INHERITANCE

UNDER the above heading, Dr. Castle reviews a paper by Yuzo Hoshino on the inheritance of the flowering time in peas and rice.

Since reading this review, Prof. Hoshino kindly sent us his paper, and we have ourselves examined it with care to see whether indeed it necessitates Dr. Castle's rather sweeping conclusions, namely, that certain genes are themselves modified by crossbreeding, one of the conclusions of Hoshino himself, and that selection within a pure line, within a genotypically pure population is effective.

It is well known that Dr. Castle counts among the few last geneticians, who still believe that the genes themselves are modifiable by selection. Hitherto in nearly all his writings on the subject Dr. Castle claimed, that unit characters vary, and may be modified by selection, a statement which can not very well be opposed, given the loose way in which the obsolete term unit character is usually applied. But it was clear, that Dr. Castle really believed the genes themselves to be capable of variability in potency, quality and value, and we think it of the utmost importance that in the review under discussion he has stated the

question in these words. Thus the issue between Dr. Castle on one side, and Johannsen and us on the other narrows, and there need be no more difficulty as to the exact meaning of the term unit character. As to the effectiveness of selection in genotypically homogeneous material, all the evidence so far adduced shows that selection in such material is absolutely ineffective. It is evident that selection in a population is usually effective, but this only shows that in ordinary populations, even in so-called pure strains of animals, there is a good deal of genotypic variation, or in other words impurity.

The fact, for instance, that Dr. Castle's selection in hooded rats was effective, shows that his material was not originally pure for all the genes. In all those instances where the guarantee for genotypic purity of the material was reasonably good, selection has, until now, proved ineffective. We need only point to the fifty years of selection in wheats by the de Vilmorin family, and to the numerous selection-experiments with clones of *Paramœcium* by Jennings and others.

As to the so-called instances of the effectiveness of selection on the genes themselves in alleged genotypically homogeneous animal material, we repeat that the only way to show such an effect in material which offers no sure proof of purity would be to change a strain of severely inbred animals by selection to a point removed from the range of the ordinary modification in the material, continuing the inbreeding, and then, by contraselection, to bring the character under consideration back to its starting-point. Since we wrote down this challenge to the believers in the variability of genes, one such a series of selection-experiments has been performed, namely, on flies, and in this series it has been proved to be impossible to get the material back to its original quality.

According to Dr. Castle, Hoshino's Table 6 shows the effect of selection within a pure line. In the cases taken from Hoshino's paper, in which the progeny of an early-flowering and a late-flowering individual of the same "pure line" can be compared (in the original table there is one more case in which the earliest parent gives the latest progeny) there are more instances in which an early parent gives an earlier progeny than a late parent, than cases in which an earlier parent gives a later progeny. But if we examine the figures more closely, we observe that the mean deviation of offspring from parents in the case in which the earlier parent gives the earlier progeny is 0.52 day,

whereas the mean deviation of offspring from parent in cases in which the earlier parent gives the later progeny is larger, 0.65 day. The only conclusion from Hoshino's Table 6 is the one he makes himself, namely, that the variation is insignificant.

We are sure that no unbiased person would conclude from the negative facts in the table in question that the variation in these pure lines was genotypic, or that selection in these groups has had an appreciable effect.

On page 332 Dr. Castle writes:

> If I have correctly interpreted Hoshino's observations, flowering time in peas is clearly a Mendelian unit character, entirely devoid of dominance, so that a strictly intermediate hybrid form is the commonest end product of a single cross between early and late varieties.

Indeed, if Hoshino's work on the inheritance of flowering-time of peas were the only, or the first, or the most comprehensive of its kind, we could see reasons for such a belief. But Hoshino only crossed two varieties differing in time of flowering. But in peas there do not exist only one late and one early variety, but several thousands, each with its own time of flowering. It would not be difficult to give a list of ten names of pea-varieties of which in every preceding one all the plants would be in flower before one of the next opened its first flower. Therefore crossing experiments involving two varieties can never be sufficient basis from which to conclude that flowering-time in peas is one thing or the other.

Tschernack, in his well-known experiments with flowering-time of peas (1911, Mendel's Festchrift), cited by Hoshino, made eight different variety-crosses. Whereas in Hoshino's work the two varieties crossed happened to be of such a constitution, that in the resulting F_2 generation there did not occur plants which commenced flowering at an earlier time than the earliest parent, or at a later date than the latest parent, in Tschernack's work such cases were met with. In Tschernack's experiment No. 81 (1906) there were in F_2 found plants flowering seven days earlier than the early parent; in experiment No. 82 (1916) even plants beginning flowering nine days earlier than the early parent. In experiment No. 81 (1906) there were also found plants starting to flower four days after the latest parent, and in experiment 38 (1902) there were plants, which did not begin to flower before the late parent had been in flower for a week.

It is perfectly clear, that a sort of blending may be the result of a difference between the parents in a number of genes, influencing the quality under observation in different directions.

On page 333 Dr. Castle writes:

In typical blending inheritance the determiners of contrasted parental conditions apparently blend into a determiner of intermediate character, the gametes formed by an F_1 individual being practically as uniform in character as those of either parent. Blending is illustrated in the inheritance of ordinary size-differences in birds and animals.

No one who knows the work of Punnett and Bailey (cited in Hoshino's paper) on chickens, in which they found not only individuals in F_2 as small as the smallest parent and as large as the largest, but even individuals lighter than the lightest parent and heavier than the heaviest, could maintain that ordinary size-inheritance in birds is blending. The gametes formed by the Hamburg × Sebright hybrids, or by our Leghorn × fighting bantam certainly were not as uniform as those of any of the four parental strains!

We are perfectly in accord with Castle when he reasons that if once we admit a contamination of genes and qualitative changes in genes, we do not need to assume that flowering-time in peas is influenced by two genes, in the cases studied by Hoshino. In such a case the difference in one gene would suffice. Indeed, we would go one step farther than Castle and declare, that, on the assumption of qualitative changes in genes, we need not assume a genotypic difference between the parent varieties at all. Where we differ from Dr. Castle is in the fact that we do not believe in qualitative variation of genes. Surely more than ten genes must influence the beginning of flowering in the pea, else there could not be so many varieties differing in the time of flowering. All the genes which influence stature, shape of flowering axis, color, must necessarily influence the onset of flowering. And we need not look for coupling between color factors and flowering-time factors, because the factors influencing color influence the metabolism of the whole plant, and thus the period at which it starts flowering.

If we compare Hoshino's paper with Tschernack's extensive experiments on the subject, we find nothing in it, which would make us assume contamination of genes by crossbreeding, or any qualitative variability of genes.

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THE SOURCES OF ANATOMICAL LITERATURE

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THE study of anatomical literature has not received the attention that has been given the writings of men in other lines of intellectual endeavor. When we compare, for instance, our knowledge of the literature of anatomy, and the men who have made this literature, with the work that has been done on the history of poetry and the poets, or fiction, or the history of nations, we see how greatly the development of anatomical knowledge and literature has been neglected. Locy[1] especially has shown us how this field of study may be used as a field of research in early human documents relating to anatomy. The subject has been further developed by Stieda, Holl,[2] Sudhoff,[3] Forster,[4] McMurrich,[5] Roth,[6] Töply[7] and others who have contributed sundry studies along these lines. That there has been no great amount of attention paid to the subject is probably due to the fact that the subject matter of anat-

[1] *Journal of Morphology*, Vol. 22, pp. 945–988, 1911.

[2] *Archiv für Anatomie und Physiologie*, Anat. Abth., Jahrgang, 1905, p. 96.

[3] Karl Sudhoff is editor of the *Archiv für die Geschichte der Medizin*, to which he is an active contributor (especially to be noted are his original contributions concerning medieval anatomical knowledge) and he is also the editor of Pagel's "Einführung in die Geschichte der Medizin."

[4] *Archiv für Anatomie und Physiologie*, Anat. Abth., 1904, pp. 372–384.

[5] *Medical Library and Historical Journal*, Vol. 4, pp. 338–350, 1906.

[6] *Archiv für Anatomie und Physiologie*, Anat. Abth., 1905, p. 79; 1906, p. 77.

[7] Anatomische Hefte, Bd. 25, Erste Abth., pp. 351–398, 1904.

omy has been and is of more interest than the form in which it is presented.

There have been a number of classical studies in the history of anatomy, the latest and best of which is that by Töply.[8] Other and earlier studies to be mentioned are the productions of Lauth,[9] Haller,[10] Portal,[11] LeClerc and Manget,[12] Tarin,[12a] James Douglas,[12b] Goelickius and many other early attempts at bringing together the results of anatomical study.

More recently the work of Daremberg in France,[13] Carus,[14] Wieger,[15] Weindler,[16] and Hopf[17] in Germany; Osawa[18] in Japan, Bardeen[19] in America, and Chievitz[20] in Denmark are to be especially mentioned.

Besides the results contained in the above-mentioned works there is much information to be gleaned from the numerous histories of medicine and especially from the

[8] Geschichte der Anatomie, in "Handbuch der Geschichte der Medizin," begründet von Th. Puschmann, herausgegeben von Max Neuberger und Julius Pagel, Bd. II, pp. 155–326, 1903.

[9] Thomas Lauth, 1815, "Histoire de l'anatomie," Strassburg. Up to the time of Thomas Bartholin, 1671.

[10] Albrecht von Haller, 1774–1777, "Bibliotheca Anatomica," Tomes I–II.

[11] Antoine Baron Portal, 1770–1773, "Histoire de la anatomie et de la chirurgie," Paris, Tomes I–VI.

[12] Daniel LeClerc and Jacob Manget, 1685, "Bibliotheca Anatomica."

[12a] Pierre Tarin, 1753, "Dictionaire Anatomique."

[12b] James Douglas, 1715, "Bibliographiæ Anatomicæ specimen, seu catalogus omnium pene auctorum, qui rem anatomicum professo vel obiter scriptis illustrarunt."

[13] Ch. Daremberg, 1870, "Histoire des sciences médicales comprenant l'anatomie, la physiologie, la médicine, la chirurgie et les doctrines de pathologie générale." 2 tomes.

[14] J. Victor Carus, 1872, "Geschichte der Zoologie, bis auf Joh. Mueller und Charl. Darwin."

[15] Friedrich Wieger, 1885, "Geschichte der Medizin und ihrer Lehranstalten in Strassburg vom Jahre 1497 bis zum Jahre 1872."

[16] Fritz Weindler, 1908, "Geschichte der gynaekologisch-anatomischen Abbildung."

[17] Ludwig Hopf, 1904, "Die Anfänge der Anatomie bei den alten Kulturvölkern." Abhandl. zur Geschichte der Medizin, Heft IX, Breslau.

[18] Gakutaro Osawa, 1895, "Zur Geschichte der Anatomie in Japan." Vortrag gehalten in der Naturforscher-Ges. zu Freiburg i.B. am 20, Nov. 1895. A.A.B. 11, N. 16/17, pp. 489–504, 2 Abb.

[19] Charles R. Bardeen, 1905, "Anatomy in America," *Bulletin of the University of Wisconsin*, No. 115.

[20] I. H. Chievitz, 1904, "Anatomiens historie," Copenhagen.

biographical dictionaries of Panckoucke,[21] Gurlt und Hirsch,[22] Pagel,[23] and the various biographical encyclopedias. Much valuable biographical data of many biologists is to be found in the "Nouveau Larousse Illustré," as well as in other general encyclopedias.

Even a hasty survey of the geographical development of anatomical literature will suffice to show that the continents of Europe and North America are the chief ones to be considered. Asia, Africa, Australia and South America each come in for some slight claim to attention, as will be evident from the discussion of the geographical distribution of anatomists given below.

It can not be said that all of the men considered in making up the list referred to below have contributed new ideas to anatomy. Many have not. None of the Romans were men of original ideas, at least so far as a knowledge of anatomy is concerned. Celsus is the only Roman whose knowledge of anatomical subjects demands any sort of respect and his knowledge, as given in the "De Medicina," is not acquired first hand although he is to be greatly respected for producing a medical classic.

Stieda[23a] has shown, however, that the Romans were not entirely devoid of anatomical knowledge, though this knowledge was often erroneous. The "Donaria" described and figured by Stieda are supposedly offerings to the deity in connection with the suppliant's plea for health. The part offered, in the form of a model of a leg, foot, breast, viscera or head, indicates the region in which the suppliant suffered and from which distress he wished to be relieved. The objects are of marble and bear the date of about the first century B.C. They are, for the most

[21] C. L. F. Panckoucke, 1820, "Dictionaire des Sciences Médicales-Biographie Médicale," 7 volumes.

[22] "Biographisches Lexikon hervorragender Aerzte aller Zeiten und Völker," 1884–88, 6 volumes.

[23] "Biographisches Lexikon hervorragender Aerzte des XIX. Jahrhunderts," 1901.

[23a] Ludwig Stieda, 1901, "Anatomisch-Archäologische Studien," II. Anatomisches über alt-italische Weihgeschenke (Donaria), Anatomische Hefte, Bd. XVI, pp. 1–84, Taf. I–IV.

part, crudely done and can not be taken as indicating any attempt to illustrate anatomy. Often the internal parts shown bear some resemblance to the human structures. Occasionally the liver of a mammal is incorporated in the same piece with the human heart and lungs. The viscera are very crude and can not be taken as indicating any degree of positive knowledge concerning the parts shown.

None of the Arabians produced original ideas or literature concerning anatomy. Abdollatif (1162–1231) is the only one of the Arabians who departed in the slightest degree from the writings of Galen and Hippocrates. While in Egypt he was studying some human bones in a cemetery when he ascertained that the jaw is formed of one piece; that the sacrum, though sometimes composed of several, is most generally of one. On the basis of these observations he criticized the writings of Galen and thus showed himself to be a man of original ideas.

Flores[24] in his "History of Medicine in Mexico" has listed the following teachers of anatomy in the University of Mexico: Febles, Benítez, García, Cheyne, Peña, García Cabezon, Rendon, Escobedo, Villar, Jecker, M. Andrade, Muñoz, Villagran, Durán, F. Ortega, Chacon, Montes de Oca, Velasco, San Juan, and Cordero é Icaza, but, so far as I have been able to ascertain, none of these men have been productive.

There is no Egyptian literature of anatomy, and apparently there was no definite knowledge of anatomical structure. The practise of embalming had attained at one period great perfection in Egypt and this may have resulted in a certain degree of anatomical knowledge, but it was so overclouded by religious fanaticism and superstition that it amounted to little. Thus McKay[24a] says: "The Egyptians probably knew next to nothing about anatomy, as their religion forbade dissection, and the embalmers probably learnt little. After the Paraschistes

[24] Francisco A. Flores, 1888, "Historia de la Medicina en Mexico desde la época de los Indios hasta la Presente," Tomo I–III.

[24a] W. J. S. McKay, 1901, "The History of Ancient Gynaecology," New York, p. 6.

had made the preliminary abdominal incision, the Tarichcutae were accustomed to pass their hands through the incision into the body and remove the heart and kidneys and digestive organs. If they were accustomed to remove the uterus and ovaries, they must have gained some knowledge of the organs, but we have no authority for saying that the uterus was really removed. The custom that the Egyptians followed, that of making models of the parts[24b] that had been healed and then hanging them in the temples, may have been useful for clinical instruction.''

Among the Hebrew peoples of ancient times sacrifices (Genesis xv. 9–10) were common, and the appearance of the viscera of the animals sacrificed were probably familiar. This may have resulted in a degree of anatomical knowledge. There are, apparently, no definite statements concerning anatomical matters in the Bible, the information there given being of a purely popular character. In the Babylonian Talmud, however, there are a number of references[25] to subjects of anatomical interest. The number of bones in the skeleton is estimated at 248 or 252, and one of these, the bone Luz, which was supposed to be situated somewhere between the base of the skull and the coccyx, was regarded as the indestructible nucleus from which the body is to be raised from the dead at the resurrection.[26] The Talmud also displays some knowledge of the esophagus, larynx, trachea, and the membranes of the brain. The pancreas and other internal organs are briefly referred to.

Among the ancient peoples who thrived in and around Mesopotamia there is, apparently, no anatomical literature. What little of anatomy may have been known was acquired through religious observances, such as auguries and sacrifices. Stieda[27] especially has studied the indi-

[24b] These were ''Donaria'' and the custom was doubtless derived from the Romans.

[25] Julius Preuss, ''Biblisch-Talmudische Medizin,'' Berlin.

[26] F. H. Garrison, *N. Y. Med. Jour.*, 1910, Vol. 92, pp. 149–151.

[27] Ludwig Stieda, *Anatomische Hefte*, Bd. 15, pp. 673–720, Taf. 57.

cations of anatomical knowledge among these ancient peoples as this knowledge has been preserved in their sculptures. It is a matter of great interest that he has interpreted a terra-cotta object from Babylon, to which an age of from 2000–3000 B.C. may be assigned, as a model of a sheep's liver, supposedly used in connection with soothsaying or with sacrifices. This interpretation is sustained by the description of two other similar objects of a later date, one in alabaster from Piacenza, and a bronze liver from Settina. One can recognize on the visceral surface of these objects the processus papillaris, the processus caudatus, and the vesica fellea; all of which are very clearly represented. The lower surface of the object from Babylon is divided into squares and studded with inscriptions, supposedly of a prophetic nature.

In addition to these very definite anatomical models many plastic representations exhibit some knowledge of the superficial musculature of the extremities. The larger subcutaneous veins, such as the cephalic, basilic, and saphenous, are often clearly shown. From an anthropological standpoint it is noteworthy that various racial types are indicated in some of the representations of the head, so that we can not say that these peoples were entirely devoid of anatomical knowledge and we are forced to admit their keen powers of observation.

Such anatomy as was taught to students in the medical schools of China was highly erroneous and fanciful. Although the study of medicine has a very ancient history in China, as ancient as the history of its civilization, going back to more than 3000 years B.C., anatomy was not studied at all in any laboratory form. They taught, for instance, that there are 365 bones in the human body; that the small intestines were attached to the heart; that they were traversed by the products of digestion; that the larynx opens into the heart; the spinal cord into the testicles, that the lung has eight lobes; the liver seven, that the kidneys, suspended the vertebral column, have the form of an egg and possess the subtle principle of generating the spermatic

fluid, primarily elaborated by the brain, condensed in the testes and from there conveyed to the spermatic duct. Osteology was somewhat better known, although the skull, pelvis, forearm and leg were regarded as being formed of one bone each, or at times eight bones were assigned to the head in the male and six in the female. Something was known of the tendons and ligaments. The spleen and heart were regarded as the organs of reason. Ancient Chinese medical literature consists of a large number of works, none of which are of any scientific importance. There is no modern Chinese anatomical literature.

What little of anatomy was known in ancient India is contained in the writings of Atreya, a physician who wrote a good description of the bones of the human body, and who is said to have taught in the Taxila University during the sixth century B.C.; as well as in the writings of the surgeon Susruta, of a somewhat later date. 'As the writings of these men have been interpreted by Charaka, ancient East Indian anatomy regarded the body of man as possessing seven skins, seven elements, 300 bones, 24 nerves, 3 fluids, 107 joints, or 68 movable joints and 142 immovable ones, 900 ligaments, 90 tendons, 40 principal blood vessels with 700 branches. and 500 muscles. The blood vessels and nerves[28] radiated out from the umbilicus as a center. Nothing was known of the courses of these structures within the body.

There is a later publication of about A.D. 800 entitled "Amarakosha," which discusses somewhat the nature of the human body but there is no later treatise which might be termed anatomical, and there is no modern East Indian anatomical literature.

While engaged in a biographical study of the men who have contributed to the advancement of our knowledge of vertebrate anatomy, the writer has been attracted by a number of interesting facts relating to the sources of anatomical literature, which, he believes, are not generally

[28] Haeser, "Lehrbuch der Geschichte der Medizin," Bd. I, p. 18–20.

recognized. The following preliminary study is an attempt to arrive at some conclusions as to the origin and development of our modern anatomical literature. It is presented here in anticipation of further studies along these lines.

The names of the men included in the above-mentioned study are of those anatomists, or contributors to anatomical literature, who are no longer living, and who have contributed in any way to the anatomy of the vertebrates, whether by practical or theoretical studies. The men of all countries and all times have been listed, so far as it has been possible to ascertain them. Doubtless many have been overlooked because their records are in relatively obscure places.

The subjects represented in the present study are: human anatomy, artistic anatomy, anthropology, comparative anatomy, embryology, histology, zoology, vertebrate paleontology and subjects of general interest which bear theoretically on the morphology and evolution of the vertebrates, such as Mendel's work at Brünn, and the work of Darwin, Weismann, Charles Bonnet and Lamarck. It may be contended that these subjects constitute biology rather than anatomy, but biology is certainly the more inclusive term. We may, to be sure, speak of the anatomy of the bacteria and in a sense the bacteriologist is an anatomist, but for my present purpose the names of those men who have contributed to our knowledge of the morphology of the vertebrates will suffice. One of the guides, which has been useful in selecting the names suited for the list, is that of the anatomical terminology. If there are anatomical structures (such, for instance, as Hesselbach's triangle) named for the man, he is included, though this is by no means the only guide. Those men whose writings are of a strictly taxonomic nature are not included, unless important theoretical results have arisen from such writings, such as has been the case with the taxonomic work of Lamarck, Linnæus, Cuvier and others.

It is hoped later to make a comprehensive survey of the development of anatomy. Over one thousand names have been compiled, and will ultimately be studied. Eight hundred of these have already been partially examined and will shortly be published.

In the development of our anatomical literature there has been involved a whole host of men in a number of different lines of activity, which it will be interesting to discuss. Many of the men included in the list were not professional anatomists but since they contributed to the advancement of anatomical knowledge we may regard them as contributors and they are hence deserving of consideration.

As would be expected, the following survey of the anatomists indicates, in general, an intellectual development in each country at the time when other conditions, physical, social, religious and political, favored the growth of mental work among the nation, although this statement finds certain contradictions, as in the case of Michael Servetus, Abano, Fallopio, Malpighi, Vesalius, and many more of the early students especially, whose work was accomplished under adverse conditions. The dates of the majority of the anatomists who find a place in the present study belong to a period when intellectual endeavor had attained a firm place in each country. Among the Greeks for instance, there is no one who attained eminence in anatomy later than the third century A.D. No modern Greek anatomist is included in the list.

There are representative anatomists of twenty-seven nationalities, though many of the countries are not widely separated geographically. The following geographical distribution indicates nothing previously unknown, but is presented here simply as an interesting survey. There are doubtless many more men of all these nations who are deserving of mention. The list contains 1 Japanese (Mitsukuri, 1858–1909, an embryologist, who is here regarded as the only one of this nation who has occupied a high place in anatomical work); possibly also the zoolo-

gist Nishikawa and the anatomist Taguchi should be mentioned; 1 Armenian (Alcana Mosali, who in the thirteenth century wrote a treatise on the anatomy and diseases of the eye, chiefly compiled from Arabian, Chaldean, Jewish, Greek and other sources); 1 Hungarian, 1 Polish, 1 South American (Florentino Ameghino, 1854–1911, a student of vertebrate paleontology, is the only representative of the large South American continent in the list. Ameghino's attainments in vertebrate paleontology entitle him to a high place among the anatomists of the world); 1 Turk (Schanzi Zadeh Mehemmed Ataullah, a Turkish physician, who after completing his studies in Italy, published, in 1820, a work on human anatomy, in folio, illustrated with 56 copper plates); 2 East Indians (Atreya, a physician, who was a teacher in the Taxila University in the sixth century B.C. He wrote an osteology, which was later edited by one of his students, Charaka. Susruta, an East Indian surgeon also deserves mention), 2 Bulgarians, 3 Flemish (of whom the greatest was Vesalius), 4 Romans (none of them men of originality), 5 Russians, 5 Belgians, 7 Irish, 7 Swedish, 7 Spanish, 7 Bohemians, 9 Arabians (Abdallatif, Albucasis, Avicenna and others), 11 Scottish, 12 Danish, 16 Swiss, 17 Austrians, 22 Greek, 36 American, 40 Dutch, 77 English, 78 Italian, 127 French and 240 Germans, making a total of seven hundred and thirty-six. The citizenship of many of the men studied has been hard to determine on account of the migration of teachers from country to country, which in the seventeenth, eighteenth and nineteenth centuries has been very common; but the above is a fair representation of the proper distribution of the men who have developed anatomy.

More than twenty-five professions are represented by the men who have been given a place in the list. In attempting to decide the position of a man in the following scheme it is not always easy, on account of the varied interests of some of them, to place them properly. Should Albrecht von Haller, for instance, be regarded as an anato-

mist, philosopher, poet, physiologist, botanist, or as an administrator, since he attained some eminence in all of these lines? Should Emanuel Swedenborg be classed as a philosopher, anatomist, geologist, civil engineer or theologian? In the present scheme Albrecht von Haller is arbitrarily regarded as an anatomist, although a very large share of his work was physiological. Swedenborg is regarded as a philosopher, for as such he is usually classed, although his anatomical writings were of a high type. Descartes is likewise regarded as a philosopher, although he might with justice be called a mathematician or anatomist. The subdivisions of histology and embryology are necessary since a few men specialized strictly in these branches of anatomical work, and they are known for their contributions to these subjects; such for instance as Balfour's noted studies in embryology and Corti's in histology.

The following list will show in a general way the numerical distribution of the men in various professions: 1 jurist, (Johannes Peyligk, who in 1499 published in Leipzig his "Philosophie Naturalis," which contains ten figures of separate organs of the body), 1 statistician, (Francis Galton), 1 beadle or exciseman (Leeuwenhoeck,[29] who for thirty-nine years worked as a subordinate customs officer or beadle at a salary equal to $125 per year. In spite of this meager income he contributed 375 papers to the Royal Society of London and 17 to the Academy of Science in Paris, besides making all of his microscopes), 1 pope (Innocent XII, who, working under the direction of Lancisi (1654–1720), is said to have been one of the first to observe, under the microscope, the circulation of blood in the capillaries); 1 prior, 1 journalist, 1 theologian (Caspar Bartholin, the founder of a professorial dynasty in the University of Copenhagen whose members taught in

[29] It should be noted that the following account is that of Sir Benjamin Ward Richardson, "Disciples of Æsculapius," Vol. 1, p. 111. Garrison, however, says in his "Introduction to the History of Medicine," p. 185, that Leeuwenhoeck was "an inheritor of well-to-do brewers (and) led an easy-going life."

the university for one hundred and thirty-five years); 1 lawyer (Michel Alberti who contributed nearly 300 separate works on several phases of human knowledge); 1 bibliographer (Mangetus, "Bibliotheca Anatomica"), 1 clergyman (Wm. Buckland), 2 monks (of whom one was Gregor Mendel and the other Michael Servetus, a Spanish monk, the discoverer of the pulmonary circulation, which he published in 1553, seventy-five years before the appearance of Harvey's great work on the motion of the heart and the circulation of the blood); 3 physicists (Helmholtz and others), 5 poets (Mark Akenside, who wrote his inaugural dissertation on the fetus; Goethe, who is widely known for his papers in comparative anatomy, and for his homology of the intermaxillary bone of men and mammals; Lucius François Anderlini, a surgeon of Saint-Angelo, in the duchy of Urbino, who published in 1739 a poetical work "The Anatomist in Parnassus, or a Compendium of the Parts of the Human Body arranged in Verse"; Scipion Abeille, a military surgeon in Flanders who wrote, in 1689, a poetical anatomy on the parts of the head and neck; Albrecht von Haller was a poet of note, and many other men, interested in anatomical subjects, have been poetically inclined), 8 artists (Albrecht Dürer is well known for a work on human proportions which is of value from an anthropological standpoint; Michelangelo, working with Realdo Colombo (1494–1559), became deeply versed in human anatomy; Leonardo da Vinci, about 1510, completed a wonderful series of anatomical sketches, based on his own dissections); 5 ophthalmologists, 5 anthropologists (Blumenbach (1752–1840) was the founder of this science); 5 comparative anatomists, 6 embryologists, 7 pathologists, 9 histologists, 8 botanists, 14 paleontologists, 17 philosophers (Aristotle, Descartes, Swedenborg, etc.), 24 physiologists, 52 zoologists, 69 surgeons, 175 physicians, and 255 professional anatomists who devoted most of their attention to the teaching of anatomy.

It has not been possible to determine accurately the

profession of many of the men included in the list, and for this reason many whose names are in the list are not classified here. For instance, Petrus d'Abano, who published in 1496 the first illustrations of the abdominal muscles, was either a physician or a professional philosopher, and probably the former, since Locy says the illustrations seem to have been based on a dissection. And there is a story concerning the large fees charged by Abano, which indicates that his profession may have been medicine, although his intellectual interests were chiefly philosophical. Bartholomæus Anglicus was probably a physician or a publisher. At any rate he published in 1485 one of the first printed illustrations (a wood cut) of any anatomical interest. Many surgeons have contributed to anatomy, and were really at the same time teachers of anatomy, such as Nicolas Ivanovitch Pirogoff, who wrote an enormous cross-section anatomy in five volumes, published in 1852; notwithstanding which he is classed in the list as a surgeon, since his anatomical teaching appears to have been incidental to his surgery.

The social status of the men who have developed anatomical knowledge has been difficult to determine because of scant biographical data. There is sufficient, however, to indicate that contributors to anatomical knowledge have been recruited from a wide range of social conditions. Some of them, and often the brightest, have lived in poverty. Others have been representatives of a much higher social class. It may safely be said that the study of biological matters has attracted attention of no special class, but that interest has been scattered. It may be said that the great majority of men who have developed anatomical knowledge have been men of moderate attainments, belonging to an average rank in the social scale. The above statements must be modified by the conditions under which the men lived and the age in which they lived. During the early centuries of the Christian Era living conditions in general were not so wholesome[30] as they have since become.

[30] Hirsch, August, "Handbook of Geographical and Historical Pathology," 3 vols.

Precocity and productiveness have gone hand in hand among the few anatomists who have exhibited these interesting traits; yet it is only fair to state that productivity has not been dependent on precocity. Such men as Bichat, Balfour, Haller, Vesalius, Johannes Mueller, Bernard Siegfried Albinus, Pollard, Sir Charles Bell, are rather unusual examples of precocity.

It may be of interest to give a few detailed accounts of some of these men. During the short period of seven years, beginning his career at the age of twenty-three, which Marie François Xavier Bichat (known as the father of histology) devoted to his scientific studies, he came to be recognized as one of the foremost biologists of all time. He exhibited unusual talents for prolonged and intense application to the pursuit of his favorite science. Besides editing the surgical writings of his teacher, Pierre Joseph Desault, in three volumes, he is himself the author of three separate works, any one of which would have secured him fame. Bichat's claim to recognition as a great biologist lies in his division, in 1800, of the tissues of the body into twenty-one non-microscopic varieties.

Francis Maitland Balfour ended his brief career at the same age as did Bichat, thirty-one; but during the few years he devoted to his favorite study of embryology he laid a secure foundation for lasting fame. Especially in his monograph on the development of the elasmobranch fishes and in his comparative embryology, he exhibited a broad grasp of the subject which has seldom been equalled in the same field of learning.

Andreas Vesalius, the great Flemish anatomist, descended from a family of learned physicians, began his study of anatomy at the age of fourteen with Dubois in Paris, and at the age of twenty-two was called to Padua to give public demonstrations in anatomy. His large work on human anatomy, "De corporis humani Fabrica," which earned him the title of the founder of modern systematic anatomy, was published when he was thirty. Although he lived for twenty years after its appearance he

did little or nothing to develop anatomy save to issue successive editions of his "Fabrica." Three years before his death there appeared from the press at Madrid his edition of Fallopio's anatomy.

Albrecht von Haller, Swiss anatomist, physiologist, poet, botanist and administrator, deserves to be regarded as the most precocious and one of the most productive of all the men who have contributed to the advancement of anatomy. At the early age of eight he is said to have compiled a biographical index of over 2,000 eminent men and women. This prodigious activity he continued for the next sixty years, and it is stated that he conducted a monthly scientific journal to which he himself contributed 12,000 articles on nearly every phase of human knowledge. Nor were his contributions superficial, for Sir William Turner says that his anatomical descriptions and his beautiful and accurate figures were the most valuable which had appeared up to that time (1746–51). A list of his medical writings alone fills eleven octavo pages of closely printed type. Late in life he returned to Berne from Göttingen, where from 1736–1753 he had held the position as professor of anatomy, physiology, surgery and botany, to engage in his native land in municipal administration.

Johannes Mueller, who, in the first half of the last century, became famed as an anatomist, zoologist, and physiologist, became, at the age of twenty-five, professor extraordinary of physiology at the University of Bonn. He began an early career of prodigious activity, which he continued for thirty-three years.

Avicenna at the age of seventeen was regarded as an excellent physician. At twenty-one he was the author of several treatises. He was called "The Prince of Arabian physicians" by his contemporaries.

Bernhard Siegfried Albinus (1697–1770), for fifty years a teacher at the University of Leyden, was called to the University at the age of twenty-one, from Paris, whither he had gone on the advice of his father, to study

medicine and especially anatomy with Winslow and Sénac. He had hoped to spend some years in Paris, but after six months, on the retirement of Rau from the professorship of medicine, anatomy and surgery at Leyden, Albinus was called, at the suggestion of Boerhaave, to take charge of the anatomy. Shortly after reaching Holland the University of Leyden gave Albinus his doctorate of medicine without either examination or thesis. His inaugural address "Oratio inauguralis de anatome comparata" clearly showed the master mind. At Leyden, Albinus gave a new direction to the study of anatomy which had lain dormant since the appearance of Vesalius's "De Corporis Humani Fabrica" (1543). He brought to greater perfection the art of anatomical illustrating, which had not progressed since Vesalius, and especially in his "Historia musculorum hominis, Leyden, 1734, in-4°", on which his fame as an anatomist rests. This magnificent work was twice reprinted and translated into French by Pierre Tarin in 1753. Albinus published also other valuable works and left a marked impression on his subject.

It would appear, from the above study, that the sources of anatomical literature are to be found in the writings of the men who have developed the subject in the various countries mentioned. The literature of anatomy has now attained sufficient dignity to warrant the preparation of a "Source Book," which would be very useful. Africa, aside from the Grecian incursion in the early centuries of the Christian era which resulted in the Alexandrian school, has produced no men of attainments in anatomy. South America has one man to its credit. Mexico and China have none. The literature of the rest of the world has radiated out from those European countries which have fostered our modern civilization. The outlook for an excellent type of anatomical literature in the future is better than it has ever been and the student who attempts to work in the field of the history of this literature will find himself among interesting and delightful surroundings.

THE CASE OF TRICHOMONAS[1]

DR. PHILIP HADLEY

The great group of flagellated protozoa has, within the past two decades, afforded a wealth of interest for those concerned with pathogenic protozoology; and only in slightly lesser degree for those concerned with taxonomic problems involving these highly interesting microorganisms. The field of trypanosome research has, in itself, afforded much new data on morphology and on complicated life histories; and has been the chief center of interest for many years.

But there exists another group of the flagellated protozoa, represented by some of the commonest forms encountered in the intestinal tract of man and the lower animals, whose frequency of occurrence, simplicity of organization and freedom from imputations of possessing pathogenic powers, have enabled them to go their way, for the most part unmolested by the protozoologist. If the protozoan would escape the inquiring gaze of the researcher he must be self-effacing; he must lead a quiet life of seclusion, free from those public manifestations of unrest and mob movement which are sure to bring him, sooner or later, before the bar of investigation, whereupon his whole life is laid bare.

Trichomonas was such a quiet law-abiding protozoan before the trouble began, before he was detected in instigating internal revolutions which bid fair to annihilate the turkey-raising industry of the country. The circumstantial evidence which has been brought forward against him has served to reveal many aspects of the life history of *Trichomonas* with which we were not previously acquainted; to disclose his participation in activities for which he was previously regarded as scarcely capable, and to demonstrate the existence of certain family resemblances to some of his companions in mischief who have long been recognized as trouble-makers in the cell organizations of many animals.

[1] Contribution No. 231 from the Agricultural Experiment Station of the Rhode Island State College, Kingston.

Trichomonas is found living in the intestinal contents of nearly all animals and has, since its discovery by Donné in 1837, appeared under many different names. It is a small organism, built on an oval or pear-shaped plan, and measuring in the adult trophozoite stage, about 10 μ in length by 5 to 6 μ in breadth. The youngest free-swimming stages are much smaller, about 5 μ in length; and sometimes trophozoites are encountered that measure 12 or 13 μ. Although usually of an elongate oval or pear shape, the morphology of the trophozoites is highly variable, and triangular or crescentic forms are frequently encountered, especially among the young. The anterior end is usually blunt, while the posterior end is frequently drawn out into a point.

If one adds to the salt solution in which these flagellates are being examined a little albumen or glycerin, to lessen the rapid swimming of the organism, some of the details of structure can be made out. The body plasm shows a greenish tint, and the nucleus, which is situated anteriorly, appears pinkish. In fresh preparations, one of the most obvious features is the axostyle, a short bristle-like structure which projects outward somewhere in the posterior quarter of the body, and which is seen, upon careful focusing, to extend into the body of the flagellate, running anteriorly to terminate somewhere in the vicinity of the nucleus (Figs. 1, 2). Inside the body the axostyle appears homogeneous in structure and bandlike.

Next to the axostyle, the most obvious feature is the vibratory or undulatory membrane which extends like a curved fin down the dorsal side of the flagellate body (Fig. 1). It is shallow at the beginning and at the end, but midway of its length it may have a depth of 2 to 3 μ. Over this membrane may be seen to travel at 3 to 4 μ intervals, waves of motion from the anterior toward the posterior end of the body. If one follows closely the course of this membrane, it is found to have its origin in a granule, or in a group of small granules, located in front of the nucleus at the most anterior part of the animal, and known as the blepharoplast-complex. The granules

are very small, measuring not more than 0.5 to 1.0 μ, and stain deeply with the chromatin stains. Their function is at the present time only a matter of speculation. Tracing the dorsal membrane posteriorly, it is found to extend to the extreme end of the body, where it narrows and is continued in the form of a terminal flagellum ("Schleppgeissel") which has a length ordinarily about equal to that of the body.

From the anterior end of the flagellate extend three more flagella (Fig. 1). These may be even longer than the body of the flagellate itself, and beat downward, as indicated by the arrow in Fig. 1. It frequently appears as if two of these three flagella were united in a common stalk at their base, so that they beat together, while the third flagellum beats independently. The origin of these three flagella is difficult to make out, but in many cases they appear to arise from one of the granules of the blepharoplast-complex, and usually not from the granule which is the origin of the undulatory membrane.

The only other structures which can be seen well in fresh preparations are the mouth or cytostome and the food vacuole. The cytostome is a horn-shaped opening which extends into the body on the ventral side, and just behind the nucleus (Fig. 2). It may be bordered by cilia. The beat of the anterior flagella is in such a direction that currents of fluid containing the bacteria which serve as the chief food for the flagellates, are driven into the mouth opening. Posterior to the nucleus, usually in about the middle of the cell body lies an oval space, the food vacuole (Fig. 2). It may sometimes be represented by a group of smaller vacuoles which coalesce to form a single cavity. In these vacuoles are usually present bacteria and cocci undergoing digestion. The structures mentioned above can be seen well in unstained organisms, but there are others which appear to advantage only upon staining. In preparations stained by the Heidenhain iron-hematoxylin method (wet process) the most noteworthy of the remaining structures is the chromatic line. This is a heavily-staining band which extends like the arc

of a circle from the blepharoplast to the point where the undulating membrane terminates. It thus follows closely, in the body plasm, the trend of the membrane, and is regarded as representing a kind of supporting structure. The chromatin line is heavier in its mid part and tapers at each end. As will be pointed out later, when in the process of spore formation, the trophozoites round off, the chromatin line becomes bent into a hoop, so that its extremities come very near to meeting (Fig. 4).

Another structure which appears with distinctness in stained preparations is the line of chromatic blocks (Figs. 3, 4). These peculiar bodies appear as a single or double row, or as a somewhat irregular line, of deeply staining granules extending from the region of the blepharoplast backward through the plasm to end somewhere in the posterior quarter of the cell. The anterior portion is likely to be thicker and sometimes may partially obscure the nucleus. The curve followed by the line of blocks is about parallel to that of the chromatic line and the two are seldom far distant from one another.

It is interesting to observe in connection with all of these struetures that, in their arrangement, they produce in the flagellate organism a more or less perfect bilateral symmetry. The normal swimming position of the trophozoite is with the undulatory membrane above. Directly below this extends the chromatic line and below the chromatic line is the "line of blocks." The cytostome is in the midline and somewhat ventral. The blepharoplast is in the midline except in some of the stages of division. The food vacuoles occupy a variable position, but are usually grouped near the middle of the posterior body and caudad of the chromatic line. Sometimes it appears as if the line of blocks and the axostyle passed through the food vacuoles. The axostyle projects from the cell body in the midline although not necessarily at the most posterior part of the body. This symmetry is easily seen when the organisms are observed swimming freely in a favorable medium. Owing to the fact that the dorso-ventral diameter is greater than the transverse diameter, most

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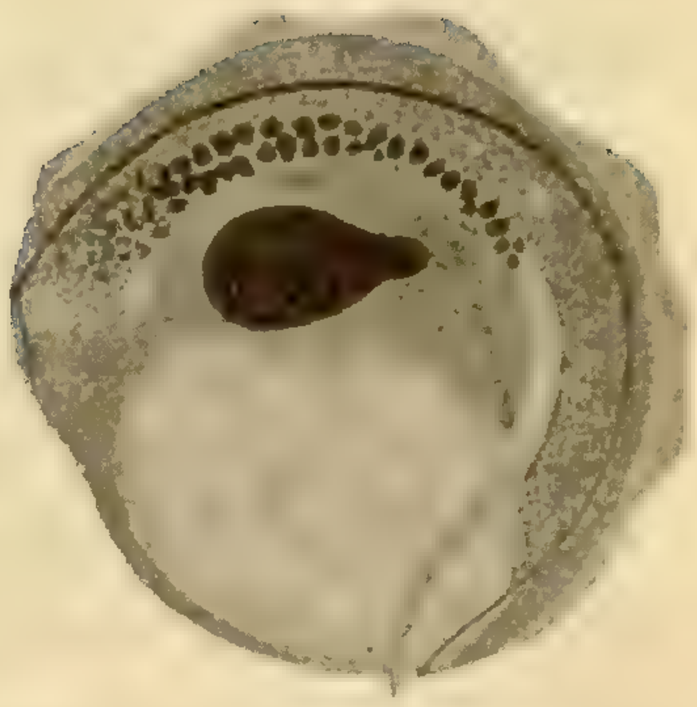

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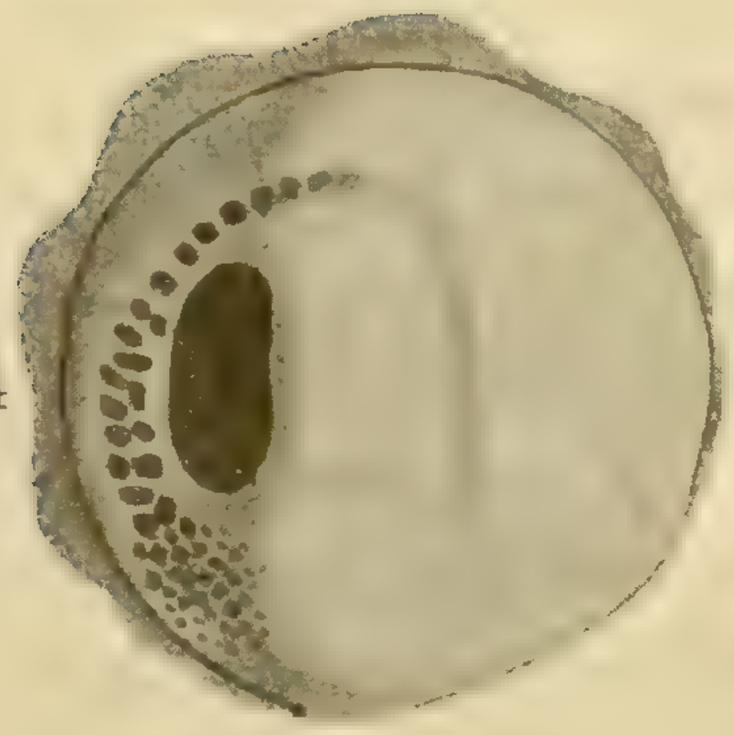

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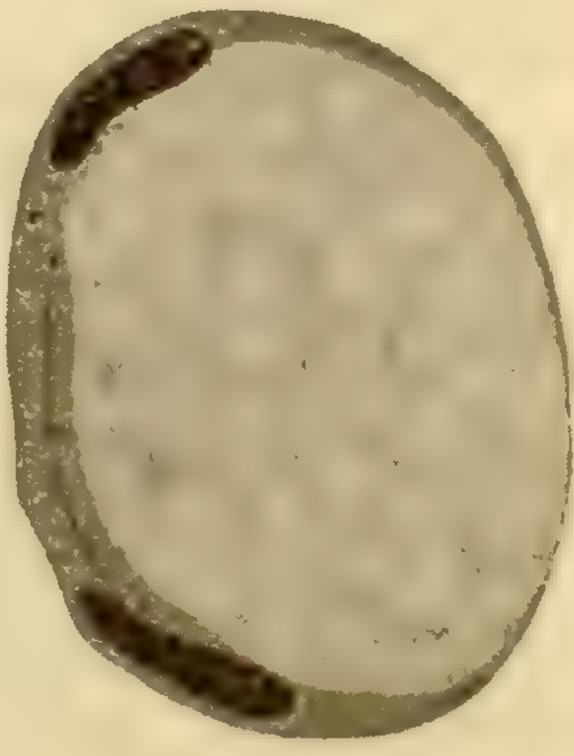

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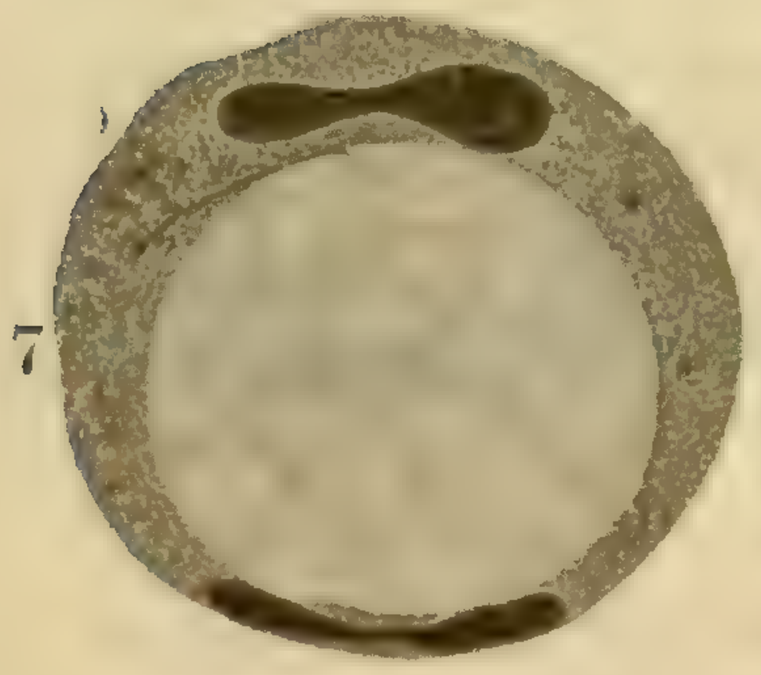

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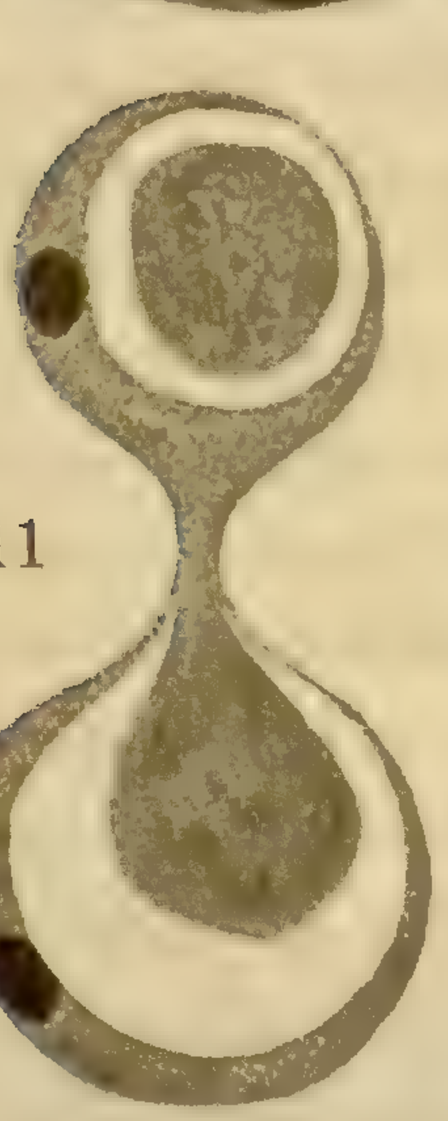

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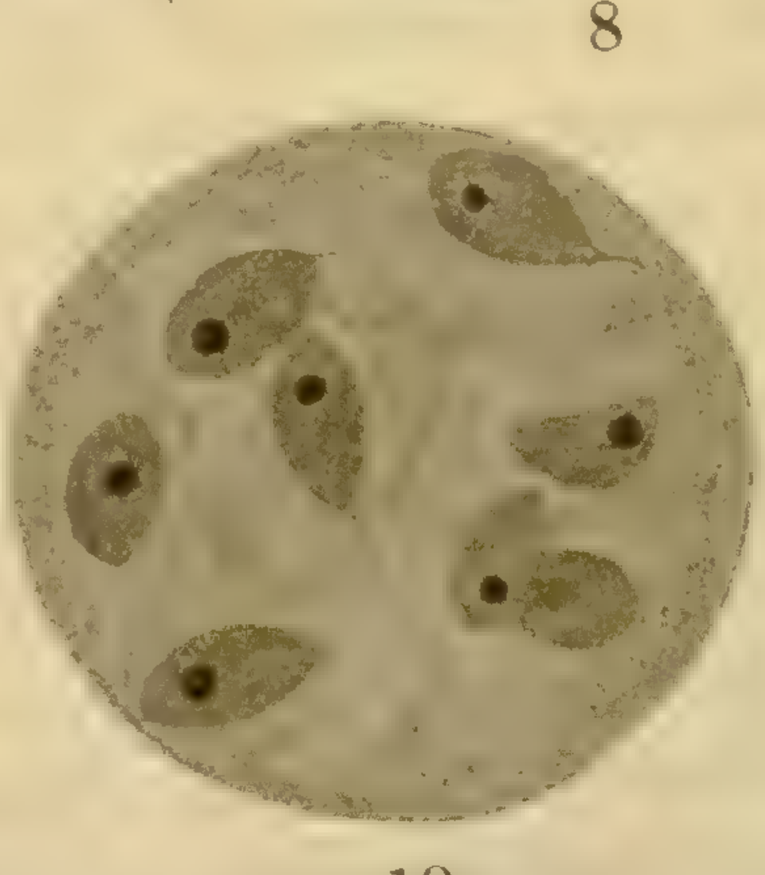

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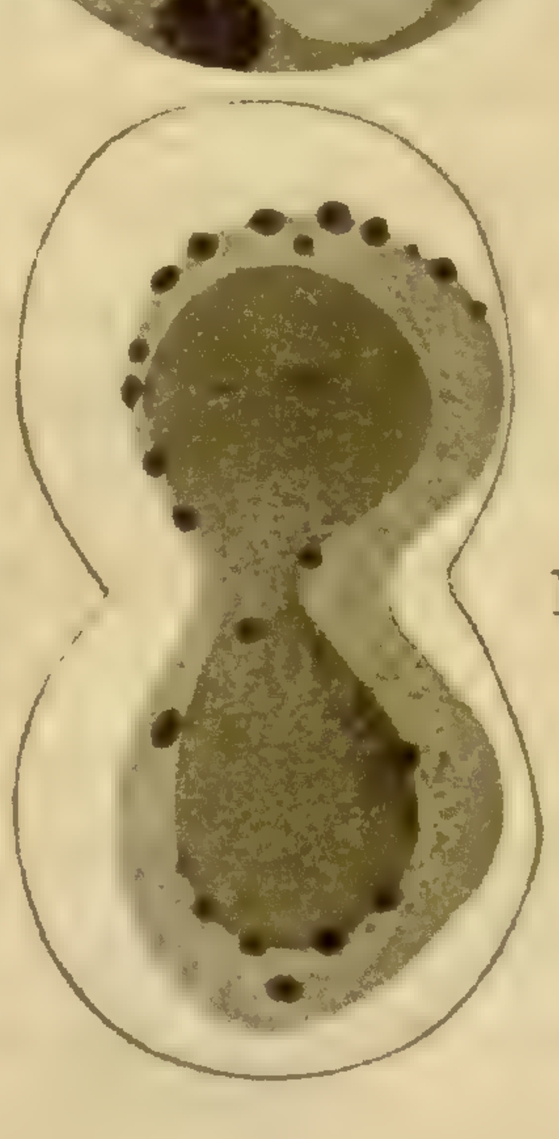

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of the flagellates when stained on the slide present a lateral aspect as shown in Fig. 2, since they fall over on to their side in the drying out of the film.

But the appearance of the flagellate as described above does not endure for very long, simply because the trophozoite stage itself does not endure. The development of the trophozoite marks the period of youth, and when the organism has sufficiently fed on bacteria and cocci, and obtained a sufficient amount of reserve food, it passes on either into division or into a form of autogamous reproduction by which the flagellate population is increased at a rapid rate.

In the case of division, the process seems to be for the most part longitudinal. The first indication of it is to be seen in the blepharoplast-complex and in the nucleus. From each new blepharoplast there appears to grow out a new chromatic line, extending more or less parallel to the old line. From these new lines the new undulating membranes appear to arise. The writer has not been able to observe the division-stages of the flagella, although stages have been seen in which new flagella are present in connection with each new blepharoplast. Neither has it been possible to follow the changes in the axostyle. As to the chromatic blocks, these also seem to disappear and are probably formed anew in the daughter cells.

But reproduction by division, though occurring commonly in the intestinal content, is probably not the chief method of reproduction. At all times, though at some times more markedly than at others, the flagellates enter into a course of autogamous reproduction in which several daughter cells are formed out of a single mother cell. This interesting process can be followed in considerable detail by means of suitably stained smear preparations.

The first step in this process is the "rounding-off" of the previously elongate or crescentic trophozoite after it has reached maturity. If the body-form was crescentic there occurs a filling-out of the concave surface so that at first a full oval shape is produced; later the organism becomes spherical. This rounding-off process, which is

usually accompanied by some increase in size, is marked by important changes in the structures alluded to above. These may be considered in some detail first with reference to the external features.

Perhaps the most noteworthy change, aside from the assumption of a spherical shape, involves the chromatic line. This gives the appearance of lengthening until it forms a hoop almost completely encircling the organism (Fig. 4). It is common to see the ends of the line occupying positions less than 45 degrees apart as measured on the circumference of the spherical flagellate. At the same time the flagella have been lost and the undulatory membrane has decreased in size, though it follows approximately that part of the circumference corresponding to the chromatic line. Of course its functioning has been proportionately reduced and although its undulatory motion may continue, this movement fails to cause progressive movement of the flagellate, but brings about a slow rotation of the organism in the same position. Occasionally this movement may be assisted by a single anterior flagellum or a remnant of one which remains after the others have disappeared. In the final stage all trace of the cytostome is lost, and in fresh preparations the organism appears as a ball of fairly homogeneous fluid, surrounded by a granular cytoplasm containing the nucleus (Fig. 5).

But more interesting are the changes that have been occurring in the internal structures, as revealed by stained preparations. The alterations in the food vacuole are possibly the most significant. In the trophozoite stage the vacuole was made up of one or more spaces representing probably not more than one eighth to one tenth of the organism (Fig. 2). As the rounding-off process proceeds, the vacuole increases in size until it occupies the greater part of the ventral portion of the flagellate (Fig. 4). It begins to crowd the cytoplasm against the dorsal wall, and in this area lies the nucleus, which, as a result of pressure, becomes somewhat flattened. At the same time the "line of blocks" and the axostyle, which gives the

appearance of passing through the food vacuole, begin to degenerate and eventually both disappear. The chromatic line endures for a longer period, however, and remnants of it may be seen for some time after the "line of blocks," axostyle and undulatory membrane have vanished. The blepharoplast also can be detected as long as the remnants of the chromatic line are visible (Figs. 6, 7). This increase in the size of the food vacuole seems to be due, partly at least, to the taking-in of fluid, since while this process is occurring the flagellate is increasing in size and becoming more plastic in the constitution of its protoplasm.

The food vacuole has now increased in size to represent the greater part of the flagellate cell and is surrounded by a crescentic ring or layer of cytoplasm seemingly much reduced in amount (Fig. 5). From this time on the most important changes concern the nucleus. This is now flattened or sometimes flask-shaped, and soon divides into two equal portions which travel through the region of cytoplasm to take positions at opposite sides of the ball of reserve substance (food vacuole). Here each experiences a further division resulting in the production of four daughter nuclei (Figs. 7, 8). These apparently may divide again until either eight or sixteen daughter nuclei are formed occupying positions about the periphery of the cell. Frequently smaller portions of nuclear substance are to be seen in the cytoplasm following the first nuclear division and it is probable that these represent reduction bodies (Fig. 8), although the writer has not observed them in the course of formation. About the daughter nuclei there seems to gather by slow degrees a layer of cytoplasm and eventually they break out of their peripheral ring of maternal cytoplasm to enter the ball of reserve substance occupying the center of the cell (Fig. 10). This is gradually consumed by the young organisms which slowly take on an elongated shape. During this time the cyst wall which had formed about the mother cell has been weakening and finally the young organisms break out of the mother cell and appear as the youngest

trophozoites measuring from 4 to 5 μ in length and about 3 μ in breadth, equipped with anterior flagella at least, and possessing a relatively large nucleus and minute blepharoplast. The other organelles characteristic of the mature trophozoites appear to develop by degrees as the trophozoite increases in size.

These, then, are the two chief methods of reproduction. Ordinarily the course is very simple, but from a study of both fresh and stained material it is clear that several complicating factors may enter. For instance there is evidence that conjugation may occur, not only between two individuals but perhaps between three or four. This process is aided by the extrusion of a viscid membrane by those organisms that have rounded-off. This naturally helps to cause the individuals to adhere together. After conjugation this viscid membrane appears to harden into a protective cyst wall. Usually the size of the single cyst is about 10 to 12 μ, but in the "fused" or conjugated forms the diameter may reach 20 to 30 μ as seen in fresh preparations. It is also clear that the "double" and "triple" cysts sometimes seen may represent a division of the original cyst, whereupon each daughter cyst continues independently the production of daughter cells by the usual method, described above.

Reproducing by the methods described above, *Trichomonas* ordinarily lives in the intestinal tract and causes no recognizable injury to the host. It has never been regarded as other than a harmless commensal. Recent studies[2] have demonstrated, however, that, upon occasion, this flagellate may depart from its usual mode of life, may penetrate the tissues of its host and cause fatal lesions, not only in the walls of the intestinal tract, but in the liver as well. It is especially this assumption of a pathogenic rôle, this sudden adaptation to a new manner of life, together with the morphological changes that accompany it, that constitute perhaps the most interesting phase of the life of *Trichomonas*. First, how does it happen that the flagellate gets started on its tissue-despoiling career?

[2] Rhode Island Agricultural Experiment Station, Bul. 166, 1916.

What is the first stimulus that creates out of a commonly law-abiding protozoan, an invader that has no equal among protozoan forms in the rapidity and completeness with which it carries on its ravages in the intestinal tissues?

This is a difficult question, and one which can not be answered with any degree of finality at the present time. The facts of the matter are these: The manifestation of the disease, as it appears for instance in the so-called blackhead of turkeys, is invariably preceded by a diarrheal condition in which the flagellates appear in increasing numbers as the course of the disease advances. Finally they appear, not only in the liquid cecal content, but in the very depths of the cecal tubules or crypts; and finally in the tissues behind the epithelial wall. From this position, by a process of autogamous reproduction, the invasion of the mucosa, submucosa, muscularis mucosæ and even the muscular layers, goes on rapidly; and eventually the whole cecal wall is crowded with the parasites. Secondary bacterial infections may intervene and the results are almost invariably fatal. The question now arises: Are these countless flagellates, present in the liquid cecal contents at the beginning of the attack, the *cause* or the *result* of the diarrheal condition? Clinical evidence, which can not now be presented in detail, seems to indicate that the latter circumstance is the actuality: that the diarrhea is the primary condition and the increase in the number of parasites the secondary. To explain the "first cause" of the disease, then, one must explain the cause of the diarrheal condition; and this, of course, is likely to prove in itself, a complex problem, but seems to lead back to certain circumstances related to the nature of the food materials and their assimilation, lying outside the province of the present paper.

For a long time it was not clear how, after their rapid multiplication in the intestinal content, the parasites were able to penetrate the epithelial wall and reach the subepithelial tissues. Recent studies[3] have shown the rôle

[3] Rhode Island Agricultural Experiment Station, Bul. 168, November, 1916.

played by the goblet or chalice cells of the crypts of Lieberkühn in this respect. *Trichomonas*, after congregating in vast numbers in the fundi of the crypts, with a consequent bulging of their walls forces its way into the goblet cells. It is not deterred by the nucleus or the cell wall at the basement end, but throws the former out of place and breaks through the latter to assume a position beneath the epithelium of the crypt. The wall having been ruptured and an avenue created to the deeper tissues, other flagellates follow by the same path until many are present between the epithelium and the basement membrane. But *Trichomonas* does not halt here. It is now filled with the spirit of the invasion and quickly pushes through the basement membrane into the loose connective tissue of the mucosa. This tissue is speedily overrun by the advancing hosts, the barrier of the muscularis mucosæ is passed and the entire submucosa exposed to the ravages of the parasite.

It is here that we recognize *Trichomonas* in a new rôle. Having experienced its first taste of blood its whole nature is changed; it becomes another animal, raging through the tissues and impeded by no protective action that the host organism is able to muster to the defense. Here then we must recognize *Trichomonas* as a cell parasite, an organism that has the power to actively invade living cells and to bring about their destruction. One may remark that the type of cell invaded is highly specialized type, and one that, by its nature, is more or less open to invasion. But the fact remains that *host cells are invaded*, and actively invaded; and in this circumstance we can detect, in the behavior of *Trichomonas*, a foreshadowing of those cell-invading activities regarded as characteristic of the sporozoa.

But of course the host-organism must put up some defense, and sometimes a very vigorous defense is offered, chiefly by means of its batteries of endothelial and other phagocytic cells. These come out in numbers to meet the invaders and as a result many of the flagellates are engulfed, either by single endothelial cells or in giant cells.

But the curious part of this circumstance is that the engulfing of the parasites seems to be of slight avail in retarding the invasion; and, in many instances without appreciable detrimental effect upon the parasites engulfed. From observations on the staining reactions and on the morphological features of the ingested parasites there is good evidence that *Trichomonas* is not disintegrated by the process; and much less killed outright. It shows a marked resistance to the plasm of the endothelial cells, within which it frequently appears that development may proceed, and from which a new generation of flagellates may break out to continue the course of infection. This would imply that the parasites, once engulfed, are able to make use of the plasm of the endothelial cell as food. And some evidence actually seems to support the view that the parasites fare better in the endothelial cells than they do without. In any region of invaded tissue the majority of the organisms are present within the engulfing cells. If these views should prove valid it must be admitted that a curious situation is produced: the parasites, to survive, must be ingested by the defensive cells, while these phagocytic agents in carrying out their normal defensive function, are favoring the growth and activity of the invaders. Of course the residence of *Trichomonas* within the endothelial cells is purely a passive cell-parasitism, although the penetration of the goblet cells is an act of active cell-parasitism. But when we regard both together, the matter is of considerable interest in its bearing upon the origin of the sporozoa, cell parasites most exclusively. From such elementary invasive power and from such primitive toleration of unfavorable host-cell influences as we see in *Trichomonas*, it is easy to imagine how the most effective stages of sporozoan parasitism may have evolved. It is a beginning of that marked adaptability of form and of physiological organization which lies at the base of all pure parasitism as it occurs in the higher orders of the protozoa.

Another noteworthy feature in the life of *Trichomonas*, and one which again serves to connect the organism with

the accepted type of sporozoan parasitism deals with the manner of obtaining its food. It has already been pointed out that when the trophozoites are developing in the intestinal contents they ingest large numbers of bacteria; whether, at this time, osmosis plays any part in cell nutrition is a question. When *Trichomonas* has entered the deeper tissues, however, the situation is different, since there are ordinarily few bacteria in these regions. Here it seems that nutrition by osmosis must play an important rôle in supporting the life of the rapidly multiplying organisms. It thus appears that *Trichomonas* is sufficiently adaptive to new conditions of existence in the tissues to substitute an osmotic method of nutrition for the ingestive. This nutrition by osmosis it will be at once recognized is one of the characteristic features of the sporozoa, and here again is to be seen a link connecting these two protozoan types.

But there is another point of interest involved in this change in the manner of nutrition when *Trichomonas* enters upon its tissue despoiling career, and this concerns the influence of the manner of nutrition upon some of the morphological features of the parasites.

In regarding the appearance of the flagellates preceding their invasion of the tissues, and after they have gained a foothold in the submucosa, a marked difference is to be observed. This has already been mentioned and may be so great as to deceive one into the belief that the parasites which are found in the intact crypts and which penetrate the epithelial wall, are not identical in nature with the organisms occurring in the deeper tissues. It is this difference which has led some writers to believe that we are dealing with two different protozoan forms. The difference lies primarily in the following circumstance: In the cecal content the flagellates are represented by two forms, the motile trophozoite and the encysted organism. In the case of the latter, one can usually observe clearly the large ball of reserve-substance, and the relatively large daughter nuclei. When developing in the tissues, on the other hand, although the motile forms can be recognized

without difficulty and although the sporulating forms, characterized by the presence of the daughter nuclei, are also observable, both of these are relatively uncommon, and the stage which shows the well rounded ball of reserve-substance ("Reservestoffsballen") is seldom met with. How can these phenomena be explained?

The writer has introduced this point in connection with the discussion of the methods of nutrition of the flagellates, simply because it seems possible that the morphological differences alluded to above are conditioned by the nature of the food supply. The writer has already traced the changes which the food vacuole of *Trichomonas* undergoes during the process of encystment. It was shown that there is a direct transformation from the food vacuole of the trophozoite, laden with bacteria and cocci, to the ball of reserve-substance which eventually crowds out the nucleus, chromatic line and line of blocks from the inner part of the cell and may possibly absorb the axostyle. Finally it comes to lie as a mass of varying size with respect to the cell, in the center, or slightly to the ventral side of the organism. Its staining qualities suggest a glycogen-like substance, and its density appears to vary with the stage of digestion of the food substances which are to serve the young daughter cells.

As stated above, this well defined reserve-substance mass is seldom observed, at least well developed, in the flagellates located deep in the tissues where the evidence favors a view of nutrition by osmosis. The question is therefore raised: Can it not be that the marked difference between the appearance of the flagellates in the tissues and in the cecal content is dependent directly upon the nature of the store of reserve food; and thus indirectly upon the manner of nutrition. This view is in agreement with the general observation that protozoa that subsist by osmosis seldom manifest either food vacuoles or definitely segregated bodies of reserve food substance.

In just what way the presence or absence of a ball of reserve-substance would explain all the differences observed in the parasites in and out of the tissues it is diffi-

cult to say. That its absence would determine a more homogeneous cytoplasm at all stages of growth is, of course, obvious, but its effect upon the cell structures such as chromatic line, blocks and axostyle, and upon the relative size and distribution of the daughter nuclei is still not clear. It can scarcely be wondered at, however, that such a radical change in the manner of nutrition of a parasite would be accompanied by alterations of some developmental significance.

Upon superficial observation it appears that, in a parasitism of this sort, when the organisms are driving ever deeper into the tissues, one of the essential features of complete parasitic activity is absent, namely, the ability to escape from the tissues and to secure a position by virtue of which the parasite can insure the possibility of reaching other hosts. Without this possibility provided for, no parasitism can be called complete. Although, in the case before us, many of the parasites are so buried in the tissues, a study of the trend of the infective process as a whole has revealed a means by which the organisms return to the cecal content after their invasive career has ended. This is by spreading downward and inward through the reticular tissue of the cores of the villi and pushing the epithelium off of the villus tips. Behind the epithelial wall at these points the parasites congregate in vast numbers until finally the epithelium breaks and liberates the flagellates into the cecal contents. That this process of escape from the tissues takes place only over certain areas of the intestinal wall is apparent; but the fact that it occurs at all is sufficient evidence to indicate that *Trichomonas* is not wholly lacking in this essential element of successful parasitism.

And finally we find in the case of *Trichomonas* one more lesson, and this is one for the etiologist, this being of course any one who concerns himself seriously with disease etiology. This important person, confronted with a disease of unknown cause, busily sets about to discover the germ; and having found the germ, he as busily engages himself in ascertaining means and measures

whereby the germ may be avoided by all susceptible folk. We are warned to avoid the places where the germ lurks, to boil our drinking water and to put cotton in our noses; and of course this has been of immense value in preventing infection in the case of many communicable diseases. But this conception of escaping the germ, a procedure still by force of habit widely applied, unfortunately does not work out successfully in all cases, simply because we have at last found that the germ is not always escapable. It may be right with us day and night; and whether we succumb to an eventual invasion depends not upon our side-stepping the organism, but upon our maintaining certain of the body defenses at the proper level of efficient working. The case of *Trichomonas* in its proper host is an instance. For twenty years (under other names) it has been consistently avoided and wholesomely feared by intelligent turkey raisers. Five hundred regulations more or less have been directed against it; and now we find that it is always there and always will be there. To keep it in an amicable state, to deter it from making destructive excursions into the tissues, all that is required is to maintain a normal and hygienic condition of the intestinal tract, whatever this may mean; this alone appears to be sufficient. Thus, although no other intestinal protozoan is able to exert, in a brief time, a greater destructive activity than *Trichomonas* when properly aroused, still we are far from justified in placing its name upon the blacklist of unqualifiedly pathogenic types which are, by both heredity and training, trouble-makers. On the other hand we can not continue to place this flagellate in that sainthood of parasites, the "harmless commensals," since, upon occasion, it may be far from harmless. *Trichomonas* must now be registered as a *facultative parasite,* which offers a wealth of interesting subject-matter for research covering several fields of biological study.

LINKAGE IN MAIZE: ALEURONE AND CHLOROPHYLL FACTORS[1]

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Genetic linkages or correlations are beginning to confirm the modern chromosome conception of heredity. As the Mendelian analysis of a species reaches a point where the known genetic factors exceed the number of chromosome pairs, certain group relations between the factors should become evident. Comparatively few genetic linkages have been observed in plants, however, probably because the number of Mendelian factors that have been determined is relatively small compared with the number of chromosomes in most species.

Physiological or morphological correlations, on the other hand, are far more common. But in the present state of knowledge they are not classified as genetic and consequently can not be used as material for determining the relationship between any series of heritable factors.

An intensive, Mendelian study of maize is gradually revealing genetic correlations. Although more than thirty definite Mendelian factors have now been determined in this species, linkages are limited in number because of the relatively large number of chromosomes (at least nine pairs).

As early as 1906, Webber noted a general correlation in maize between color in the aleurone layer and in the stamens, glumes and silks. At that time, the genetic constitution of color in the kernels and other parts of the plant was unknown. Consequently the correlation was not analyzed on a factorial basis.

In 1911, Emerson described an apparent linkage between color of cob, pericarp, husks, silks, and anthers in

[1] Given before the Botanical Society of America at the annual meeting held in New York City, December 28, 1916. Paper No. 58. Department of Plant Breeding, Cornell University, Ithaca, N. Y.

maize. Further evidence (unpublished), however, has led Professor Emerson to prefer a simpler explanation for this phenomenon than linkage.

Collins and Kempton (1911) and Collins (1912) have reported a genetic correlation between aleurone color and endosperm texture. In this case one of the pairs of aleurone factors is apparently linked with one pair of endosperm factors (horny and waxy). The data indicate that there is a little less than twenty-five per cent. crossing over.

In a later paper, Collins (1916) describes five character pairs in maize that show apparent genetic correlation. But since these characters exhibit "blending" inheritance and have not been analyzed from any factorial standpoint, it becomes impossible to use these correlations in determining the group relations between the genetic factors concerned. Collins also notes a large number of physiological correlations in this article.

LINKAGE BETWEEN ALEURONE AND CHLOROPHYLL FACTORS

A definite linkage has been found in maize between one of the five pairs of aleurone factors (*Aa, Cc, Rr, Pp, Ii*)[2] and one pair of chlorophyll factors of which there are at least seven, as the writer has determined. Manifestly this is not a physiological correlation, for it is difficult to conceive of an anthocyanic pigment, limited to a single layer of cells in the grain, being caused by the same physiological factor that produces a plastid color, like chlorophyll, so widely distributed in the plant. Breeding evidence demonstrates that the correlation is genetic, as will be seen later.

The aleurone factor concerned is the *R* factor, which together with *C* and *A* is needed to produce any color in the aleurone cells of the corn grain (Emerson, 1917). To determine linkages with any of the five aleurone factors, it is obviously desirable to use material that is homo-

[2] Reported in a paper given before the Botanical Society of America at the annual meeting in New York City, December 28, 1916, by Professor R. A. Emerson. The paper is now in manscript form under the title, "A Fifth Pair of Factors for Aleurone Color in Maize."

zygous for as many of the non-linked factors as possible. Fortunately, the plants used in this experiment were homozygous for *A*, *P* and *i*, as will be shown later. This left only *C* and *R*, giving either 3:1 or 9:7 ratios of purple to colorless grains. The aleurone segregation on the ear proved to be exceptionally distinct because the factors *A* and *P* were homozygous.

The other factor involved in the linkage is concerned with chlorophyll development in the mature plant. It is one of at least seven factors necessary for the production of full, normal green color in maize, and it has been termed the *G* factor. Its allelomorph *g* produces a distinct yellow or golden color in the leaves and stalk of the mature corn plant. This color is comparable with that of the familiar golden-leaved shrubs. It has been described and its inheritance discussed by Emerson (1912) and Miles (1915). Suffice it to say that it is a simple recessive to normal green.

During the summer of 1914, a green plant heterozygous for *R* and *G* was pollinated by a golden plant that lacked aleurone color. The cross can best be described by the following factors:

$$\frac{GgRrCCAAPPii}{3472\ (11)} \times \frac{ggrrCcAAPPii}{3468\ (10)}.$$

Proof for the correctness of these formulæ will be given later.

A selfed ear of the female parent bore a 3:1 ratio (271:88) of purple to colorless grains, showing that only one aleurone factor was heterozygous. The male parent was likewise selfed and showed no aleurone color. On the ear of this cross there were 67 purple and 55 colorless grains, approximating a 1:1 ratio.

The purple seeds on this ear were planted separately from the colorless seeds. From the field counts of this planting, which are given in Table I, it is seen that the 1:1:1:1 ratio of independent inheritance is noticeably modified.

The observed numbers in the four classes give a ga-

metic ratio of 3.9 : 1, or 20.4 per cent. of crossovers. It is evident that the actual results agree very closely with the theoretical expectancy on a 4 : 1 basis of linkage. Indeed, the goodness of fit (Elderton, 1901 and Harris, 1912) is so perfect that $x^2 = .317$, giving a very high value for P.

TABLE I

SHOWING THE F_1 DISTRIBUTION FROM THE CROSS

$$\frac{GgRrCC}{3472\ (11)} \times \frac{ggrrCc}{3468\ (10)}$$

	From Purple Seeds		From Colorless Seeds	
	Green (*RG*)	Golden (*Rg*)	Green (*rG*)	Golden (*rg*)
Field counts	30	9	6	28
Observed, corrected for aleurone ratio[3]	28.1	8.4	6.5	30 0
Theoretical, on a 4 : 1 gametic ratio...	29.2	7.3	7.3	29.2

From these results it may be said that, at gametogenesis, the factors of the female parent are so linked that the gametes *RG* and *rg* are produced about four times as often as the crossover gametes *Rg* and *rG*. Thus far, no evidence is available for demonstrating the linkage of *Rg* and *rG* ("repulsion" between *R* and *G*).

Additional evidence on the linkage was afforded when the female parent of the cross and four other plants, in which the *R* and *G* factors were heterozygous, were selfed. Each of these plants bore ears with 3 : 1 ratios in aleurone color (total, 1052 purple : 339 colorless). The results appear in Table II.

Obviously this F_2 distribution does not resemble a 9 : 3 : 3 : 1 ratio of independent inheritance. The agreement between the actual results and the theoretical on a 4 : 1 gametic ratio, however, is close, *P* being .6733, which is considered a good fit. It may be concluded, therefore, that the factors in these five F_1 plants were so linked that *R* and *G* occurred together in one chromosome, while *r* and *g* were located in the homologous chromosome and

[3] This correction is applied to equalize the proportion of the plants from the purple and the colorless seeds. The proportion should be 1 : 1 in this case, but variation in the percentage of germination or in the number of purple and colorless seeds actually planted, often disturbs it. Such a correction is of course legitimate.

that crossing over occurred about twenty per cent. of the time.

TABLE II

SHOWING THE F_2 DISTRIBUTION WHEN F_1 PLANTS HETEROZYGOUS FOR *R* AND *G* WERE SELFED

Selfed Plants	From Purple Seeds		From Colorless Seeds	
	RG	*Rg*	*rG*	*rg*
3472 (11)	48	4	12	24
3347 (2) parent of above	35	3	8	6
3954 (1)	5	0	2	2
3954 (3)	10	0	2	3
3954 (11)	8	3	2	6
Total	106	10	26	41
Correction for aleurone ratio[4]	125	12	18	28
Theoretical, 4 : 1 basis	120.8	16.5	16.5	29.2

When plants that were heterozygous for *G* and for both *R* and *C* were selfed, the ratio between the four classes in the next generation appeared rather unusual. This was found to be due to the influence of the 9:7 ratio in aleurone color of each of the four plants that were selfed (total, 477 purple:387 colorless). The data from such plants are arranged in Table III, which follows:

TABLE III

SHOWING F_2 DISTRIBUTION WHEN PLANTS HETEROZYGOUS FOR *G*, *R* AND *C* WERE SELFED

Plants Selfed	From Purple Seeds		From Colorless Seeds	
	Green	Golden	Green	Golden
3347 (1)	32	3	11	4
3347 (3)	79	6	51	31
3347 (4)	96	6	47	40
3477 (2)	38	3	19	11
Total	245	18	128	86
Theoretical 4 : 1 basis	236	32	122	87

There is considerable deviation from the theoretical expectancy in this case and the value for *P* is small ($P = .0808$). Nevertheless, these results, taken in con-

[4] The correction is applied because at planting time no attempt was made to plant three purple grains to one colorless grain.

junction with those in Tables I and II, accord with the idea of linkage between *R* and *G* on a 4:1 basis, especially since they deviate so widely from the 27:9:21:7 ratio of independent inheritance.

Another source of evidence on the genetic interrelations of the *R* and *G* factors was noted in a back cross in which the *I* factor for aleurone color was involved. No aleurone tests have been made, but evidence from related plants in pedigree cultures makes it seem reasonable that the following factors are concerned:

$$\frac{GgRrccAAPPIi}{3021\ (1)} \times \frac{ggrrCcAAPPii}{3018\ (10)}.$$

In this case, the ears of both parents showed no aleurone color. The ear from the cross gave a distinct segregation of purple and of colorless grains (36 purple:195 colorless), approximating the theoretical 1:7 ratio. Assuming the factors as given above, this aleurone ratio is reasonably close to the expected proportion, the numbers being relatively small. As a check upon this aleurone ratio, it might be mentioned that six of the F_1 plans from purple seed were selfed. Each one showed a 9:7 ratio of purple to colorless grains on the ear (total, 1441 purple:1088 colorless). Also three F_1 plants from colorless seed were selfed and the ears showed no aleurone color.

The field counts of the plants from purple and from colorless F_1 grains are classified in the following table:

TABLE IV

SHOWING THE F_1 DISTRIBUTION FROM THE CROSS

$$\frac{GgRrccAAPPIi}{3021\ (1)} \times \frac{ggrrCcAAPPii}{3018\ (10)}.$$

	From Purple Seeds		From Colorless Seeds	
	Green	Golden	Green	Golden
Observed...	12.0	3.0	8.0	22.0
Correction for aleurone ratio	4.5	1.1	10.4	29.0
Theoretical, 4:1 basis	4.5	1.1	18.0	21.4

Here again, while the value for *P* is relatively small ($P = .1171$), it is noted that in general the observed re-

sults correspond with the theoretical when a linkage on a 4:1 basis is assumed. Considering only the classes from purple seeds (first two columns in Table IV), the agreement is perfect. These classes are not disturbed by the aleurone situation and consequently ought to show the gametic ratio directly.

Determination of the Aleurone Factor concerned in the Linkage

It has been shown from four independent sources that the *G* factor is linked with one of the five aleurone factors, which has been termed the *R* factor. It now becomes necessary to prove that it really is the *R* factor that is involved. From the aleurone ratios observed in this experiment, as well as from evidence from related plants, it is certain that the inhibiting factor *I* is lacking, with the exception of the last cross described (Table IV). It is also obvious that the *P* factor, which produces purple color when *C*, *R* and *A* are present, must be homozygous (*PP*) in both parents of the first cross since the color in the colored grains is a deep purple with no trace of red. This leaves only *C*, *R* and *A* to be considered.

In order to facilitate the presentation of the proof that the *R* factor is the one concerned, Table V has been prepared. In this table, the zygotic formulæ of the F_1 plants of the first back cross (see Table I) are listed and the plants that were tested are noted in the last column. The factors are merely assumed as here given.

TABLE V

Showing the Zygotic Formulæ of the F_1 Plants of the Cross 3472 (11) × 3468 (10)

Parental Formulæ: GgRrCCAA × ggrrCcAA

F_1 Zygotes	F_1 Aleurone Color	F_1 Plant Type	F_1 Plants Selfed (Pedigree No. 3954) Individual Plant Nos.
GgRrCCAA	Purple	Green	(1) (3) (11) (31) (33)
GgRrCcAA.....	"	"	(34)
ggRrCCAA.	"	Golden	
ggRrCcAA.....	"	"	(32)
GgrrCCAA	Colorless	Green	(44)
GgrrCcAA.....	"	"	
ggrrCCAA	"	Golden	(21) (22) (29) (43) (45)
ggrrCcAA	"	"	

The fourth column shows the plant numbers of the F_1 plants, which were self-fertilized. In addition, plants (31), (32) and (34) were crossed with certain aleurone testers of known constitution provided by Professor R. A. Emerson.

All the grains on the ears of plants (21), (22), (29), (43), (45) and (44) were colorless. The aleurone counts on the ears from purple seed are arranegd in Table VI.

TABLE VI

SHOWING THE ALEURONE COUNTS ON SELFED EARS OF F_1 PLANTS FROM THE CROSS 3472 (11) × 3468 (10)

Ped. 3954 Plant No.	3 : 1 Ears		Ped. 3954 Plant No.	9 : 7 Ears	
	Purple	Colorless		Purple	Colorless
(1)	230	83	(32)	66	65
(3)	303	88	(34)	172	163
(11)	248	80			
(31)	180	45			
(33)	224	79			
Total	1,185	375	Total	238	228
Theoretical	1,170	390	Theoretical	262	204

These ratios indicate that plants (1), (3), (11), (31) and (33) are heterozygous for one aleurone factor only. Plants (32) and (34) also contain that factor but apparently they have in addition another aleurone factor, which likewise is heterozygous because the aleurone ratios, although they deviate somewhat from the 9:7 proportion, can not be classified as 3:1 or 27:37 ratios.

Only tests with plants of known aleurone formulæ will determine whether it is the *C*, *R*, or *A* factor that is linked with *G*. Such tests have been made by using the aleurone testers described by Emerson (1917). These aleurone testers possess colorless grains in which all the aleurone factors except *P* are homozygous. For example, the *R* tester has the formula *rrCCAAii*, the *C* tester the formula *RRccAAii*, and the *A* tester the formula *RRCCaaii*. In Table VII are presented the data involving the various tests for the aleurone factors. Plants (31), (32) and (34) possess all the three aleurone factors in question

(*R*, *C*, and *A*), as can be seen from their records in Table VI. Plant (41) is a golden type from colorless seed.

TABLE VII

SHOWING THE TESTS FOR THE PRESENCE OF ALEURONE FACTORS

Crosses with Aleurone Testers	Aleurone Color of F_1 Grains
1. *A* tester (*RRCCaa*) × 3954 (32)	450 purple
2. *C* tester (*RRccAA*) × 3954 (32)	72 " : 69 colorless
3. *R* tester (*rrCCAA*) × 3954 (32)	7 " : 11 "
4. 3954 (34) × *C* tester (*RRccAA*)	205 " : 217 "
5. 3954 (31) × *R* tester (*rrCCAA*)	212 " : 249 "
6. 3954 (41) (*rrCcAA*) × 3954 (31)	92 " : 101 "

The data in Table VII prove several things, namely:

1. That the factors *A*, *C* and *R* must be present in all the plants tested, because color was produced in some of the F_1 grains of each cross.

2. That it is the *R* factor that is linked with *G*. This is demonstrated in the fifth cross. Plant (31) was a green plant from purple seed bearing an ear with a 3:1 aleurone ratio, showing that it was heterozygous for the one aleurone factor that is involved in the linkage. The test clearly proves it to be the *R* factor, the aleurone ratio from the fifth cross approximating the theoretical 1:1 proportion.

3. That the assumptions made in Table V as to the genotypic formulæ of the F_1 plants are correct. This statement follows from a series of deductions. First, one aleurone factor of the three concerned must be homozygous (dominant) in all the plants, because the aleurone ratios observed permit only two heterozygous factors at the most (9:7 ratios). Since the *C* factor is heterozygous in plants (32) and (34), and the *R* factor heterozygous in plant (31), while *A* is homozygous in plant (32), it is obvious that only the *A* factor could be homozygous (dominant) in all the F_1 plants. Second, one of the remaining factors (*C* or *R*) must be homozygous (dominant) in some plants and heterozygous in others to account for the 3:1 and the 9:7 ratios, respectively. The second and fourth crosses in Table VII indicate that *C* is heterozygous in plants (32) and (34), whereas the fifth

cross, together with the 3:1 ratio of the selfed ear, shows that *C* is homozygous in plant (31). Third, the remaining factor, *R*, should occur only in a heterozygous or in a homozygous recessive condition to account for the 1:1 aleurone ratio on the F_1 ear of the original back cross. The fifth cross in Table VII proves that *R* is heterozygous in plant (31), and the sixth cross shows that in plant (41) this same factor is homozygous recessive.

From this series of interrelations it is seen that the hypothesis is verified in all cases and that it is the *R* factor for aleurone that is linked with *G*.

ON THE QUESTION OF CROSSING OVER IN THE MALE AND FEMALE

An interesting observation regarding the question of crossing over in plants can be derived from some of the data presented. It has been shown that crossing over occurs in gametogenesis of the female (Table I). Does it take place in the formation of the male gametes as well? In certain animals, crossing over seems to be limited to one sex. It occurs only in the female of *Drosophila* (Morgan, 1915) and only in the male of the silkworm (Tanaka, 1914). Castle (1916) has noted that the phenomenon occurs in both sexes of the rat. Among plants, the studies with sweet peas and Primula indicate that crossing over is not restricted to one sex. Perhaps it is to be expected that, in the case of most plants, where the pistillate and staminate parts are borne on the same individual, there should be no difference in the genetic behavior of the two reproductive systems in this respect. Nevertheless, it is interesting to note the condition in the monecious corn plant.

From the data in Table II, it is possible to demonstrate that crossing over is found in both sexes of maize. In order to do this, the observed frequencies can be compared with the theoretical expectation when crossing over occurs in both sexes and when it takes place only in the female. Such a comparison is arranged in Table VIII, which follows:

TABLE VIII

A COMPARISON OF THE RESULTS FROM TABLE II WITH THE THEORETICAL EXPECTATION WHEN CROSSING OVER OCCURS IN BOTH SEXES AND WHEN IT OCCURS ONLY IN THE FEMALE

	RG	*Rg*	*rG*	*rg*	*P* Goodness of Fit as Compared with Observed
Observed (from Table II)	**125**	**12**	**18**	**28**	
Theoretical, when crossing over occurs in both sexes	120.8	16.5	16.5	29.2	.6733
Theoretical, when crossing over occurs only in the female	128.0	9.0	9.0	37.0	.0067

Clearly, the first theoretical expectation fits the case adequately, for with such a high value for P it is almost certain that the deviations of the observed results from the theoretical are due to errors of random sampling only. Consequently one is justified in saying that crossing over occurs in both male and female. This is especially true when the fit in the second case is so poor. In fact, the great difference between the two values for P makes it seem reasonable that the intensity of the linkage is equal in both male and female, although the high value for P in the first case suggests that directly.

ADDITIONAL LINKAGES BETWEEN CHLOROPHYLL FACTORS AND ALEURONE

Preliminary tests indicate that the same chlorophyll factor, *G*, that is linked with *R*, is also concerned in a linkage with one of the other chlorophyll factors termed *L*. The latter has been found to be one of three factors, two of which have already been described by Miles (1915), involved in the production of chlorophyll in the seedling stage of maize.

Factors *G* and *L* seem to be linked although the data from three back crosses, in which the numbers are small, exhibit some variation in the percentage of crossing over. Details of this linkage will appear in a later paper, dealing with the inheritance of the three seedling chlorophyll factors.

Apparently, then, the factor pairs *Rr*, *Gg*, and *Ll* con-

stitute one factorial group in maize. It is to be expected that *Rr* and *Ll* should bear a definite relationship to one another. This has not yet been fully determined, although there are some indications of such a linkage, for aleurone color and chlorophyll development appear to be genetically related in different manner from that noted previously.

When purple seed of certain ears are planted separately from the colorless ones, the former give a distinct segregation of green and white seedlings, while the latter give rise not only to the green and white, but also to a constant proportion of yellow seedlings. The writer has determined that these yellow seedlings depend upon a definite genetic factor. Over two thousand seedlings have now been grown and not one yellow seedling has resulted from the purple grains. Discussion of this linkage will also be reserved for the later paper, or until the aleurone factor concerned has been identified. This may be the *R* factor showing its theoretical relationship to the *L* factor.

SUMMARY

1. Linkage between the *R* aleurone factor and the *G* factor for chlorophyll development shows approximately 20 per cent. crossovers.

2. Crossing over takes place in both male and female gametogenesis of the monecious maize plant.

3. Preliminary tests indicate that *G* is also linked with *L*, a seedling, chlorophyll factor. Consequently the factor pairs *Rr*, *Gg* and *Ll* constitute one factorial group in maize.

To Professor R. A. Emerson, of Cornell University, who so generously has shared his material for this investigation, the writer is deeply indebted and desires to express his sincere gratitude.

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SHORTER ARTICLES AND DISCUSSION

THE APPLICATION OF CORRELATION FORMULÆ TO THE PROBLEM OF VARIETAL DIFFERENCES IN DISEASE RESISTANCE: DATA FROM THE VERMONT EXPERIMENTS WITH POTATOES

THE ultimate practical object of any study of disease resistance in a series of varieties is the selection for future cultivation of the few which are least susceptible. In practise a relatively large series of varieties or strains is taken into cultivation for preliminary study. The size of the cultures of the individual strains must, on a given area, be inversely proportional to their number. Since the individual cultures are necessarily small, it is impossible to assert from the results of a single test that the observed differences between the strains really represent varietal differences in disease resistance. They may be due merely to inadequately large cultures or to imperfectly controlled experimental conditions. It is therefore necessary to repeat the experiment another year or in a different locality in order to determine whether the observed differences are really persistent, and so characteristic of the strain, or whether they are due to transient conditions only. The problem is then purely and simply one of correlation. This is obviously true whether one chooses to avail himself of the advantages of the statistical formulæ or not. If the correlation between disease incidence in cultures of the series of varieties grown in different years, or places, be zero, the varieties show no permanent differentiation in disease resistance. If the correlation has a significant positive value it indicates at once that there are really inherent varietal differences in disease resistance. The numerical magnitude of the correlation indicates something of the extent of this differentiation. If the correlation be low, the prospect of isolating varieties sensibly more resistant that the average will be slight. If the correlation be high, it should be relatively easy to secure highly resistant strains.

Since the correlation method seems to have considerable value in the analysis of data of this kind, I have thought it might be of service to geneticists and plant pathologists to illustrate it by the constants which I have found it necessary to deduce for another purpose from the published records of the series of experiments on disease resistance in varieties of potatoes carried on during

[1] Stuart, W., "Disease Resistance in Potatoes." *Bull. Vt. Agr. Exp. Sta.*, 179, 1914.

the past several years at the Vermont Agricultural Experiment Station.

In a recent bulletin Stuart[1] summarizes the data obtained during five years' observations on percentage infection by early blight (*Alternaria solani*). In his Table I he gives the estimated percentage infection in a series of varieties during the period. Since during a portion of the experiment all the varieties were not considered, I have calculated the correlations in two groups. In one case $N = 149$, in the other $N = 50$. The smaller group comprises only varieties also included in the larger. The correlations, calculated by the usual product moment method[2] without grouping, appear in the accompanying table.[3]

CORRELATION FOR VINE RESISTANCE TO EARLY BLIGHT IN TWO YEARS

Years Compared	Series of 149 Varieties	Series of 50 Varieties
1905–1906	$+.055 \pm .055$	$-.056 \pm .095$
1905–1907	$+.438 \pm .045$	$+.420 \pm .079$
1905–1908	———	$-.021 \pm .095$
1906–1907	$+.042 \pm .055$	$+.226 \pm .091$
1906–1908	———	$+.323 \pm .085$
1907–1908	———	$+.082 \pm .095$

Only 2 of the 9 constants are negative; these are insignificant in comparison with their probable errors. All the constants which have substantial values and are materially larger than their probable errors are positive in sign. The average of the two negative constants is $-.038$, of the seven positive coefficients $+.227$, and of all the (unweighted) values $+.168$. Thus there is clearly a measurable differentiation of the varieties in respect to susceptibility to *Alternaria*.

The values are, however, exceedingly variable, ranging as they do from $-.056$ to $+.438$. The great variation in the actual constants I am inclined to attribute to (*a*) the difficulty of estimating the percentage of infection, (*b*) the unavoidable experimental errors associated with relatively small cultures, and (*c*) the wide variation in average percentage infection from year to year. Both (*a*) and (*b*) are factors which tend to render the actually recorded percentages somewhat erroneous as measures of the real susceptibility of the variety, and tend in consequence to dilute the strength of the correlation. With respect to the third

[2] AMER. NAT., 44: 693–699, 1910.

[3] The chief discrepancy between the results for the larger ($N = 149$) and the smaller ($N = 50$) series of varieties is to be seen in the interrelationship for 1906 and 1907 where the two correlations are $.042 \pm .055$ and $.226 \pm .091$. Here the disagreement is apparent rather than real. The difference is $.184 \pm .106$, which can not be considered statistically trustworthy.

factor, (c), it is obvious that if there be only a very slight average percentage infection the test of disease resistance will not be a very critical one, whereas the average percentage can not be very high indeed unless conditions are so unfavorable that all varieties are affected. The percentage infection of early blight varies enormously from year to year. Thus:

	Percentage Infection	
Year	$N = 149$	$N = 50$
1905	3.3	2.4
1906	85.0	83.2
1907	41.7	38.9
1908	—	10.8

With an incidence of 3 per cent. one year and of 85 per cent. the following season one can, in view of the considerations mentioned above, hardly expect to obtain smooth values of the correlation coefficient.

It is interesting to compare these results with those for other maladies of the potato. In the same publication Stuart gives the results of trials for resistance of tubers to scab. Unfortunately the experiments of the second year, 1907, included only 20 of the 65 varieties from the first year. Calculations may be based on the percentage of tubers which are free or nearly free from scab. This is much lower the second year.

	1906	1907
Mean	64.21	28.20
S. D.	11.59	16.33
Correlation	$r = .591 \pm .098$	

The probable error is high because of the fewness of the varieties retained in the second year's test, but the correlation is of more than medium value and is relatively about 6 times as large as its probable error. Thus susceptibility to scab is probably to a very considerable extent a varietal character.

The results for tuber rot tests are not available for successive years, but Stuart has given[4] the percentage of tuber rot in 89 varieties grown on sandy loam and on clay loam soil in 1905. For these I find

	Sandy Loam	Clay Loam
Mean	8.68	39.76
S. D.	11.28	26.85
Correlation	$r = .653 \pm .041$[5]	

[4] Stuart, W., "Disease Resistance in Potatoes," *Bull. Vt. Agr. Exp. Sta.*, 122, Tables VI–VII, 1906.

[5] The value of r given as $.707 \pm .045$ in *Science*, N. S., 38: 402–403, 1913, is deduced from the 62 varieties for which laboratory cultures were available, and from modified percentages. The values agree within the limits of their probable errors.

The correlation of these data, somewhat smoothed by Jones,[6] with a series of determinations of the percentage growth of the fungus on tubers *in the laboratory* has already been determined.[7] For laboratory growth and loss on clay loam, $r = .584 \pm .059$. For laboratory growth and loss on sandy loam, $r = .594 \pm .055$.

Taken as a whole these correlations indicate (*a*) that susceptibility to both early and late blight and to scab differs greatly from variety to variety, and (*b*) that, so far as the evidence goes, the varieties differ more in resistance to tuber injury than to foliage infection by early blight.

It is not at all necessary that the correlations be drawn between the amount of injury to the same organs of the plant or by the same disease. In many instances the so-called cross correlations yield valuable results.

For example Stuart[8] discusses the question of the relationship between vine infection and tuber rot. The point may be subjected to a statistical test by correlating between the maximum percentage of foliage affected by late blight as given in his Table V for potatoes grown on sandy loam soil in 1905 and percentage of rot as recorded in his Tables VI and VII. Unfortunately, the percentages are available for the vines for sandy loam soil only (Table V) while the figures for tuber rot are given for both sandy loam and clay loam soil. Both correlations may be worked out. I find:

For percentage foliage infection on sandy loam soil and per cent. tuber rot on sandy loam soil

$$N = 131, \quad r = .316 \pm .053.$$

For percentage foliage infection on sandy loam soil and per cent. tuber rot on clay loam soil

$$N = 80, \quad r = .102 \pm .075.$$

In both cases the correlations are positive, and hence such evidence as they furnish indicates that the varieties which show the greatest infection of the leaves actually are the worst to rot. That the correlation between injury to the tops and tuber rot is higher on the sandy loam soil is not at all surprising, since the same individual plants—not merely the same varieties—are in-

[6] Jones, L. R., N. J. Giddings and B. F. Lutman, "Investigations of the Potato Fungus *Phytophthora infestans*," *Bull. Vt. Agr. Exp. Sta.*, 168: 74–81, 1912.

[7] Jones and collaborators, *loc. cit.*, and the Reviewer, *Science*, N. S., 38: 402–413, 1913.

[8] *Bull. Vermont Agr. Exp. Sta.*, 122, p. 116.

volved in the correlation. The problem is, however, a complicated one and much more extensive data are needed for a complete analysis.

A problem of very great biological interest as well as of practical importance is that of the specificity of disease resistance. Concretely: Do varieties differ in their susceptibility to a specific disease only, or do they differ merely in susceptibility to disease in general?

A comprehensive and final answer will require far more data than are available and more stringent statistical analysis than can be illustrated here. Some progress can be made by the method of correlation as follows.

If susceptibility be purely *specific* there should be no correlation between the incidence of disease x in year (or culture) p and disease y in year (or culture) q, although there should be a correlation between the incidence of disease x or disease y in different years or cultures. If, on the other hand, differences in disease resistances from variety to variety are determined solely by general weakness or vigor of the stocks, one should expect the correlations between the incidence of different diseases in different years or cultures to be (within the limits fixed by the errors of measurement and the probable errors of random sampling) as high as those between two series of determinations of incidence of one and the same parasite.

Consider first the relationship between the percentage of foliage injury by early blight in 1905, 1906, and 1907 and the percentage of tuber rot in 1905. The correlations are:

Foliage Injury	Per Cent. Tuber Rot on Sandy Loam, 1905 $N = 131$	Per Cent. Tuber Rot on Clay Loam, 1905 $N = 89$
Early Blight, 1905....	$.167 \pm .057$	$.256 \pm .057$
Early Blight, 1906....	$.211 \pm .056$	$.249 \pm .067$
Early Blight, 1907....	$.291 \pm .054$	$.440 \pm .058$

Without exception the correlations are positive in sign. While numerically low, the most of them taken individually may be considered statistically significant in comparison with their probable errors.

Thus it seems clear that the varieties with foliage most injured by early blight are also most subject to tuber rot, just as has been shown to be the case in foliage and tuber infection by late blight.

For foliage infection by early blight in 1905, 1906, and 1907 and foliage injury by late blight in 1905 I find:

	Correlation, $N = 131$
Early Blight, 1905, and Late Blight, 1905..	$-.066 \pm .059$
Early Blight, 1906, and Late Blight, 1905..	$+.190 \pm .057$
Early Blight, 1907, and Late Blight, 1905..	$-.040 \pm .059$

The results are not so consistent as those of the preceding table. The two negative coefficients are insignificant in comparison with their probable errors, and the positive one is not large, either absolutely or relatively. Possibly the laxness of the correlation is in part due to the fact that the measurement of both characters is subject to a large possible error.[9]

For freedom of the tubers from scab with the incidence of other diseases every possible correlation has been determined. The coefficients are shown in the accompanying table. Note that in this case the correlation is between *freedom* from one disease and *occurrence* of another disease. Hence a negative coefficient has the same meaning as a positive one in the foregoing discussions.

FOR PERCENTAGE OF TUBERS FREE OR NEARLY FREE FROM SCAB IN 1906 AND 1907 AND INCIDENCE OF OTHER DISEASES

Diseases Compared with Freedom from Scab	Correlation 1906	Correlation 1970
Per Cent. rot on Sandy Loam Soil, 1905	$-.136 \pm .077$ ($N=74$)	$-.357 \pm .132$ ($N=20$)
Per Cent. rot on Clay Loam Soil, 1905	$-.280 \pm .096$ ($N=42$)	$-.030 \pm .187$ ($N=13$)
Top Injury by Late Blight, 1905	$+.035 \pm .084$ ($N=65$)	$-.224 \pm .151$ ($N=18$)
Top Injury by Early Blight, 1905	$-.561 \pm .054$ ($N=74$)	$+.240 \pm .142$ ($N=20$)
Top Injury by Early Blight, 1906	$-.119 \pm .077$ ($N=74$)	$-.352 \pm .132$ ($N=20$)
Top Injury by Early Blight, 1907	$-.118 \pm .077$ ($N=74$)	$-.040 \pm .151$ ($N=20$)

Because of the small number of varieties involved and the roughness of the measurements the correlations are low and irregular. In ten cases the negative sign indicates that the varieties which are most free from scab are also least susceptible to attacks by other diseases. In neither of the two cases of positive correlation is the constant statistically significant in comparison with its probable error.

Thus altogether 23 of these cross correlations—that is correlations between injury to different organs by the same disease, or

[9] In one case, 1905, the correlation is between foliage injury by two different organisms *in the same year*. What interrelationship is to be expected in such case requires further consideration.

to the same organ by different diseases, or to different organs by different diseases—have been worked out. Only 4 of these—that is only about one case out of six—are exceptions to the rule that varieties which show more than the average amount of injury by one disease will, on the whole, show more than the average injury by another disease. No one of these exceptional constants can be considered significant with regard to its probable error. Several of the 19 which indicate the rule may be looked upon as individually trustworthy. Thus notwithstanding the large variations in numerical magnitude incident to small series of data and rough measurement, the determinations taken collectively certainly furnish highly convincing evidence that to a considerable extent susceptibility to disease is general rather than specific.

The fact that the series of correlation coefficients here presented justify much more definite conclusions than those who have considered the data without statistical analysis have drawn, is sufficient indication of the usefulness of the biometric method in the preliminary stage of disease-resistance experiments in which large numbers of strains are being tested, and in which the mass of data is highly confusing. The special cases illustrated by no means exhaust the possibilities of the biometric formulæ now available. Had the data been more extensive, the analysis might have been carried much farther.

Nothing that has been said in this paper in emphasis of the statistical method must be taken to imply that the most careful individual analysis is not desirable and essential. The two methods are not mutually exclusive, but supplemental.

J. ARTHUR HARRIS

THE DIFFERENT MEANINGS OF THE TERM "FACTOR" AS AFFECTING CLEARNESS IN GENETIC DISCUSSION[1]

IN the analysis of alternative (or segregating) heredity, we find that certain potentialities, such as that of producing a certain color in some part of the soma, appear to be inherited independently of certain other potentialities. We assume that the germ-plasm carries various corresponding genes, factors, or determiners, whose independence in gametogenesis determines the independence of the somatic characters. Cytological study leads

[1] Paper No. 39, University of California, Citrus Experiment Station, Riverside, California.

to some very probable conclusions as to the time and method of segregation of these determiners; though it does not yet enable us to identify with absolute certainty the actual physical unit of segregation, the cytological and genetic evidence indicates strongly that such a unit exists.

In the current chromosome hypothesis, as developed especially by Morgan and his collaborators (Morgan, Sturtevant, Muller, and Bridges, 1915), the material unit of segregation is assumed to be a part of a chromosome. Breaks in two homologous chromosomes at meiosis, with consequent exchange of parts by the pair, presumably occur at certain definite points only. How close these points may be we can not say, but the general stability of Mendelian characters indicates that the number of points is limited. On these assumptions, the portion of a chromosome between two adjacent points of possible breaking is the ultimate physical unit of genetic segregation—essentially a *locus* as defined by Morgan (1915, p. 419).[2]

It is now widely recognized that, in effect, a single real unit of segregation may influence very diverse characters of the soma, often in ways which can not be at all inferred one from another. Very possibly one physical unit of segregation may affect, say, flower color and height in ways just as distinct physiologically as may two distinct units of segregation, although transmission is different in the two cases. In the latter case we say that two genetic factors are concerned; are we compelled in the former case to admit only one?

As a matter of fact, as will be evident on further consideration, either course is possible, according to the definition of *factor* accepted.

If by "factor" we mean a *developmental potentiality*, the delimitation of a particular factor is largely a matter of convenience in analysis. On the other hand, if the term is used to designate a supposed actual physical unit of segregation, a factor has a definite objective extent.

The former view is that of the presence-and-absence terminology, as it is generally understood at present. In this sense, a factor is not an element of the germ-plasm; it is rather a property or characteristic of the germ-plasm or of some element of the germ-plasm. The characters of an organism, as Gates (1914,

[2] Though Morgan, Sturtevant, Muller and Bridges (1915, p. 155) suggest the possibility that the loci of linked factors may be so near together in a chromosome "that they never (or very rarely) cross over." The definition of *locus* is discussed below.

p. 269) has remarked, are "attributes," no more to be separated from the organism than are the properties of a chemical compound from that compound. The factors of the presence-and-absence scheme, similarly, are inferred *properties or attributes of the germ-plasm*, by whose behavior we explain the alternative transmission of certain properties or attributes of the soma.

Obviously an organism is not composed of "characters"—and neither is its germ-plasm composed of "factors," so long as the factors are those of the presence-and-absence scheme. Such factors are nothing but *characters of the germ-plasm*, and, like the characters of the soma, they are more or less conventionalized in description. We have no warrant for projecting these conventionalized descriptions back into the actual germ-plasm, and assuming the presence and absence there of strictly corresponding material units of segregation.

The presence-and-absence scheme, when not encumbered with non-essential hypotheses, is a strictly neutral instrument of genetic analysis. If there is segregation in the formation of the germ-cells, it is merely a matter of definition to state that a factor is allelomorphic to its absence. That is, the assumption of segregation is the only assumption required by this scheme, which is the logically simplest form of the "conceptual notation" (East, 1912) of genetics.

Very special emphasis must be placed on the fact that the "absence" is absence of a *potentiality*, without reference to the presence or localization in the germ-plasm of any other potentiality that may actually take its place. The allelomorphism of the presence-and-absence notation is a *logical* opposition; when it makes "A" and "no-A" allelomorphs, this involves no assumption as to what may be physically opposed, in the chromosomes, to the physical basis of "A."

If an "absence" a of a given factor A is always or commonly accompanied by an actual presence of a corresponding factor A', and we wish to represent this fact, it is provided for by the terminology of linkage; we may use Aa' and aA', since the presence-and-absence scheme makes no assumptions as to the structure of the germ-plasm. It is obviously simpler to write simply A and A', or A and a, for the two factors, and convenience may justify this practise; we should note, however, that in thus abandoning the presence-and-absence terminology we introduce a second assumption, that of actual factor-to-factor opposition or allelomorphism. This assumption is, of course, in view of all the evidence, a highly probable one, and especially convenient

in cases of apparent multiple allelomorphism (Morgan, Muller, Sturtevant, and Bridges, 1915, chap. 7). In fact, this added assumption probably permits a more direct and therefore practically simpler representation of the actual course of segregation. Whether the corresponding factor-to-factor notations now used for Drosophila (Castle, 1913; Morgan, Sturtevant, Muller, and Bridges, 1915, p. 233) are everywhere adequate and convenient is another question, as Emerson (1913) has shown.

Cases of multiple allelomorphism involve no special difficulty *in principle* for the presence-and-absence scheme. They can of course be represented only by linkage formulæ, in which the "presence" of one factor of the set is linked with the absence of the rest—but their very occurrence suggests that we might, as is suggested above, correctly enough represent single factorial differences in the same way. All this can affect only the convenience of the notation, and not at all its logical applicability.

If we adopt a factor-to-factor system of notation, it is natural to conceive of the opposed "factors" not as mere potentialities, but as physical units responsible for genetic potentialities. When we have taken this viewpoint, we have begun to use the word *factor* in the second sense mentioned above; we are thinking of assumed physical units of segregation, not merely of observed potentialities of development. Morgan (1915, p. 419), in discussing "presence and absence," uses *factor* in this sense, as do Morgan, Sturtevant, Muller, and Bridges (1915, pp. 220–222).

No doubt what has been said above is an old story to experienced geneticists in general, in view of such discussions as those of East (1912) and Morgan (1915). The distinction is so fundamental, however, and the double use of the term *factor* so increases the difficulties of the case, that consideration of the general problem from the present terminological viewpoint seems highly desirable. Perhaps greater precision in the use of several terms could be attained.

Johannsen (1909, pp. 124–125), in defining the term *Gen* (gene), makes it perfectly plain that he means the material basis[3] or cause (the *Anlage*), of whatever sort, of a "unit character," defining a *unit character* as one dependent on a special kind of gene. He is evidently inclined to consider the gene as the material unit of segregation, holding that the sum of the genes constitutes the germ-plasm, and there is a widely prevalent

[3] Or *immaterial* basis, if we must admit the theoretical possibility of the existence of immaterial "entelechies" associated in some more or less mystical way with the germ-plasm.

tendency among geneticists to use *gene*, and its synonyms *factor* and *determiner*, in this sense, which is the second of the two discussed above. Evidently *gene* is not properly used in the first sense. A moment's thought, however, will show the impracticability of confining *factor* to that sense; the meaning of this term shifts back and forth continually in common usage, and often remains indefinite.

Further, when *factor* (or *gene*) is used in the second sense, we consider it coextensive with *locus* (Morgan, 1915, pp. 419–20; Goodspeed and Clausen, 1917, p. 32). A factor is a particular state or condition of a locus. Let us, then, define *locus* as the physical unit of segregation, almost certainly identified as a genetically indivisible portion of a chromosome. Genetically inseparable ("completely linked") potentialities, then, belong to the same locus, and hence to the same factor or gene; "completely linked factors" are mainly[4] relegated to non-cytological discussion, and especially to use with the presence-and-absence terminology.

No doubt Mendelian analysis considers, in any case, only some of the most readily identifiable properties of the real units of segregation concerned, and this fact seems to deserve a large place in our genetic thinking. Especially is it important that the two meanings of *factor* and its synonyms should be clearly distinguished; when these meanings are unconsciously interchanged and confused, vagueness and misunderstanding are sure to result.

The student of genetics may read, for example (East, 1912),

> If we forget ourselves and begin to speak of unit factors as particles, only a confusion follows similar to that caused by Nägeli, Spencer, and Weismann. Nothing is gained and even facts are obscured.

On the other hand, he will find the factors of *Drosophila* located with mathematical exactness in diagrams of the chromosomes, and often apparently or explicitly considered as material components of the chromosomes. In the interest of clear thinking, especially in the case of beginners and casual students in the field of genetics, the explanation of this apparent contradiction deserves very special emphasis.

As an example of the way in which this terminological conflict may cloud an argument when the essential facts are clear to the writer, we may take the following case. Morgan (1913,

[4] Obviously, temporarily complete linkage of genes must be excepted, as in the usual case with the male of *Drosophila*. The point is that the chromosome theory may well consider, in any case, that potentialities *always* associated are manifestations of the same factor or gene.

p. 10), in urging his substitute for the presence-and-absence scheme, largely on grounds of convenience, agrees with East (1912) as to the general usability of the latter scheme as a "system of nomenclature" without cytological implications. He makes especially plain the undesirability of interpreting "absence" as a physical absence in the germ-cell, or (1915, p. 419) as "a hole in a chromosome." In one respect, however, Morgan's discussion seems less clear than it might be, and this is in the use of *factor*, in these articles, in a sense (the second here) which is not that of the presence-and-absence scheme, with only vaguely implied explanation of the distinction. It certainly *is* permissible to speak of "the absence of a factor from the germ-plasm," *if we mean the kind of "factor" implied by the presence-and-absence terminology.*

We must make it as clear as possible that *factor* (1) sometimes means a potentiality and (2) sometimes means a body, and that a factor is assumed to be paired either (3) with its absence or (4) with another (identical or different) factor. The combination of (1) and (3), then, gives the presence-and-absence scheme, while the combination of either (1) or (2) with (4) gives the scheme used by Morgan (1913; see also Castle, 1913) and other students of *Drosophila*. General objections to Mendelian analysis have been based largely on confusion of (1) and (2), which often leads to erroneous suppositions—for instance, that Mendelian analysis in general, or the presence-and-absence method of Mendelian analysis, requires the unnecessary and unwarranted assumption involved in the combination of (2) and (3). Probably this confusion is also largely responsible for the persistence of another often discredited notion, the idea that "Mendelians" suppose their factors to be individually the basis of somatic characters, rather than simply necessary elements in an interacting complex which produces the characters.

Summary

The term *factor* has, in genetic use, two distinct meanings, which are continually interchanged or combined and often confused. It is essential to clearness in genetic discussion that these two meanings should be carefully distinguished. These meanings may be indicated by the following formal definitions:

1. A genetic (Mendelian) factor is a property or characteristic of the germ-plasm, more or less conveniently delimited for the purpose of analysis of segregating heredity.

2. A genetic (Mendelian) factor, or gene, is an actual material

unit of genetic segregation; it is of unknown nature, but probably consists of a genetically indivisible portion of a chromosome (a *locus*) in a particular state.

The presence-and-absence scheme of factor notation properly employs only the first of these meanings; the Morgan-Castle scheme, on the other hand, may use either.

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THE SELECTION PROBLEM

UNLESS history fails to repeat itself, geneticists, whose attention is focused upon variation, should sooner or later overemphasize its importance as a factor in shaping the organic complex. There is, indeed, reason to believe that already a tendency for some among them to do so is becoming apparent. Dr. Pearl's[1] recent paper under the title above affords an example in point.

In that communication its author fails to discriminate sharply between two distinct phases of his subject. Whether selection may affect the course of evolution is a matter entirely apart from the possibility that it alters the germ plasm. Racial history may possibly be modified, if the genetic composition of a mixed population may be affected by selection based upon somatic dif-

[1] Pearl, Raymond, 1917, "The Selection Problem," THE AMERICAN NATURALIST, Vol 51, pp. 65–91.

ferences. But modification of the germ plasm by selection is impossible, if that agency acts only as "a mechanical sorter of existing diversities."

The present note is not concerned with the causes of variation. It refers only to the first-mentioned phase of the selection problem. It accepts the statements in the quotation below as substantially correct, and attempts in brief compass to evaluate the arguments by which Dr. Pearl supports his position regarding them.

By transposition of a few phrases his ideas may be expressed in his own words as follows:

> The mere fact of elimination and survival . . . is capable, in theory at least, of bringing about evolutionary changes of a progressive sort, . . . if the elimination be selective, and the survivors transmit to their progeny those differences that mark them off from the eliminated. The theory that these two rules are always and everywhere in operation, taken together with the observed fact that living creatures do die, *is* the Darwinian theory of Natural Selection as a factor in organic evolution.

If, as is implied, Darwin gratuitously assumed the intolerable burden involved in the use of the words, *always* and *everywhere*, it is immaterial. It is not a vital issue whether the form in which he expressed himself will bear literal interpretation, but merely whether his idea is correct that natural selection effects notable changes in the course of evolution. Hence it seems sufficient to say, that if the "Dance of Death" is governed in general, or even in part, by the joint action of the two principles enunciated in the preceding paragraph, the changes described there should follow as surely, although more slowly, than if the conformity were complete.

It is stated by Dr. Pearl, as one of three broad facts on account of which natural selection is no longer regarded as a primary, or perhaps even a major factor in evolution, that even when selective elimination on the basis of somatic characters does occur, it does not follow generally and regularly that the somatic differences on which the selection acted will reappear in the progeny, . . . actual experience having abundantly demonstrated that a very great many of such somatic differences are not inherited.

This may refer, first, to the fact that a single phenotype may include members of different genotypes. Yet, even so, no strict limitation is placed upon the possibility of changing the character of a mixed population by selection based upon somatic qualities. If plus variants of an inferior strain seem superior to

individuals of a higher order of genetic worth, this will have its due effect in impeding progress; but since the essential point is, that, collectively considered, members of different pure lines do, in general, rather definitely reflect their different germinal constitution, advance may undoubtedly be made. However, it is unnecessary to labor the point, as it is not only admitted, but is urged by geneticists themselves in explanation of such results, for example, as those Castle and Phillips[2] obtained in selection experiments with hooded rats.

Reference to the genetic behavior of such characters as side-sprig of the comb in poultry may also be included in the quotation above. If, in this case, emphasis is laid upon the fact that selection might apparently be exercised indefinitely without the least tendency toward evolution of a side-sprigged race, it needs only be pointed out that the argument is directed against the contention that selection is capable of modifying the germ plasm. It has no bearing whatever upon the possibility of its occurrence in nature, nor, aside from the point indicated, upon its influence in evolution.

Upon the other hand it seems quite impossible that even if characters of which side-sprig is representative were highly useful, they should fundamentally modify the course of evolution under selection, or place any notable obstacle in its way, for they are not germinal variations and are as likely to occur in one line of descent as another. Hence, other things being equal, it is quite as probable that they should assure the survival of a subnormal representative of a superior genotype, as that they should tide over a superior representative of an inferior one; and the chance would be no greater that the attribute in question should appear in the offspring of one rather than in that of the other.

A second of the three general groups of facts to which reference has been made is summarized in the statement:

> Observation indicates that in many cases evolutionary changes have come about by relatively large, discontinuous steps, the new form being not merely fully differentiated at its first appearance, but also fully able to survive.

In another connection it is stated forcefully by Dr. Pearl that if the game of survival is actually played by the quoted rules he formulates (and no others are necessary) the conclusion is logically irresistible that progress is bound to occur in the direction of those differences which distinguish the survivors. But

[2] Castle, W. E., and Phillips, John C., 1914, Carnegie Institution of Washington, Publication 49. Castle, W. E., 1916, "Genetics and Eugenics," Harvard University Press, Cambridge, 8vo, pp. vi + 353.

since obedience to his two rules is not in the least contingent upon the magnitude of the variations upon which selection is based, it must be admitted that the facts summarized above are entirely irrelevant to the present discussion. They neither bear upon the phenomena of inheritance, nor add anything to our knowledge of selective elimination.

The third general fact cited in support of Dr. Pearl's contention regarding the diminished esteem in which natural selection is held as a factor in evolution is:

> All organisms possess in varying, but usually in very large, degree the power of personal, immediate, individual, somatic adaptation to the environment.

It is affirmed in addition, that in consequence of this power of personal adaptation the survival expectation of an individual is not generally and regularly a function of any static, single-valued relation between its somatic structure, habits or physiology, on the one hand, and the impinging environmental stresses on the other. Yet, it is asserted, such a relation is implicitly assumed in that part of the theory of natural selection which affirms a selective elimination on the basis of somatic characteristics.

The reply to these various statements is, that their substantial truth may be admitted without the possibility that evolution is affected by selective elimination being thereby in the least diminished. The adaptive capability of any individual either rests upon or lacks a germinal basis. In the former case there is no obvious reason why it should not itself provide material upon which selective elimination might be based, with consequent change in the composition of the population. In the latter, individual adaptability is as incapable of exercising influence upon the course of evolution, as side-sprig should be, if it were a useful character of the same order of importance.

If the accumulated results of genetic research provide no more effective arguments than these, it must remain an open question whether natural selection is not a primary factor, not in the *origin* of species, but in the determination of the elements composing the flora and fauna of the world at any period in its history. In other words, one who wishes to force an abandonment of that position must demonstrate that selective elimination does not occur upon such a scale that it may account for the results ascribed to it.

For many legitimate reasons Dr. Pearl has not treated this point at length in his article under discussion. But from the scanty evidence available he derives the following conclusion:

In some cases natural elimination is certainly in some degree selective, while in other cases it certainly is not, and in the most favorable cases of all the selection is apparently not very rigorous. Gross teratological abnormalities are eliminated. But the smaller deviations from type, which in theory ought to furnish the basis of selection, appear upon quantitative study less generally and sharply determinative of survival than might have reasonably been expected theoretically.

It is perhaps worthy of note that ever since it appeared that the larger, and rarer, discontinuous variations are in no danger of being lost through swamping, it has been beside the mark to ascribe especial theoretical significance to smaller deviations from type. Attention may also be directed to the important admission that in some instances elimination is known to be selective. It will then be in order to examine reports of researches upon the basis of which it is confidently asserted that in other cases the same is not true.

It happens that with five others one is cited which falls squarely within my own field of investigation. This is Professor Reighard's[3] "Experimental Field Study of Warning Coloration in Coral-reef Fishes," which Dr. Pearl seems to consider of particular significance for his own argument. But since it has been my good fortune to study the same material in the same place, more extensively and with better facilities, I feel justified in saying that Reighard's results will bear no such interpretation as is here placed upon them. He proved that gray snappers possess powers of discrimination and of memory which would lead one to suppose that, if the bright colors of the smaller reef fishes possess a warning significance, the snappers should be aware of it and avoid them. He did not prove beyond possibility of doubt that they do attack such fishes freely, but that is of no importance in the present connection; his conclusion that tropical fishes are not warningly colored with reference to their commonest enemies is perfectly sound. His ideas regarding immunity coloration are, however, the only ones in his paper which have the remotest bearing upon the matter of selective elimination, and there is no reason to suppose that these are more than logical deductions from incorrect premises.

Dr. Pearl's[4] own report upon the natural elimination suffered

[3] Reighard, Jacob, 1908, "An Experimental Field-study of Warning Coloration in Coral-reef Fishes," Carnegie Institution of Washington, Papers from the Tortugas Laboratory, Vol. 2, pp. 257–325.

[4] Pearl, Raymond, 1911, "Data on the Relative Conspicuousness of Barred and Self-colored Fowls," THE AMERICAN NATURALIST, Vol. 45, pp. 107–117.

by barred (Plymouth Rock) and black, or near-black, chickens living under the same conditions is also mentioned. In this paper photographs show the black fowls looming up against natural backgrounds much more distinctly than do the barred. This is accepted as "objective and unbiased evidence regarding the relative conspicuousness of the two types of plumage pattern." It follows naturally, since the extensive record shows little difference in the rate of elimination of the two sorts of birds, that "the relative inconspicuousness of the barred color-pattern afforded its possessors no great or striking protection against elimination by natural enemies." But photographs serve as *accurate* measures of the conspicuousness of the fowls in the eyes of color-blind enemies only. Therefore, if rats and predaceous birds are not color-blind, and there is perfectly good reason for supposing that creatures lower in organization than either have color vision, it is not at all certain that the elimination in the two cases does not correspond fairly well with the actual difference in conspicuousness of the two types.

Kellogg and Bell's[5] interesting "Studies of Variation in Insects" deals with 24 species. In 23 of them, including the lady-beetle, *Hippodamia,* to which Dr. Pearl refers, the authors show that there is much variation in individuals which have successfully run the gauntlet of natural selection. But since they have no knowledge whatever of the variation in the original populations, of which they have studied survivors only, these their results show nothing regarding the extent, or even the occurrence, of selective elimination.

In the honey bee alone duplicate studies were made of the variation of certain structures in individuals which were about to hatch, and in others, apparently from the same hive, after exposure to the vicissitudes of an active life. Among 200 drones in the first group the veins of the fore wings in 11 were imperfectly developed, and as a result normal flight became difficult or impossible. The variation in others of the first series seems essentially the same as that observed in the 300 members of the second, among which none of the defective individuals were found. But if these facts prove anything, it seems to be that selective elimination does occur when unfavorable variations affect the normal functioning of an organ. Suggestion is entirely lacking that the mechanical efficiency of the wing is impaired by the other variations noted, and it can scarcely be con-

[5] Kellogg, V. L., and Bell, R. G., 1904, "Studies of Variation in Insects," Proc. Washington Acad. of Sciences, Vol. 6, pp. 203–332.

sidered a pregnant fact, in the present connection at least, that indifferent variations provide no basis for selective elimination.

In the same two series of bees the variation in the number of hooks upon the costal margin of the hind wings was determined without significant difference appearing in the two cases. In this instance, since the hooks appear to operate to the insects' advantage in binding the fore and hind wings of each side together, it seems plausible enough at first glance, that the more hooks there are the more efficiently their function will be discharged. Upon second thought, however, a difficulty suggests itself. The number of hooks varies from 19 to 29 in different individuals, but even the smaller number may, for all that is known to the contrary, perform perfectly the function ascribed to them. In that event the others are superfluous and the advantage they confer entirely fictitious. But waive the objection, and what follows? Simply a conclusion which in its relation to the present argument is already invalidated: Variations of the *magnitude* indicated provide no "handle" for natural selection.

It is unnecessary to carry the examination of the evidence farther. The three papers which have been reviewed are not carefully selected for criticism, but are the last, and apparently the most important, of six certified to be "fairly representative." If, however, this characterization is correct, it is apparent that the case against selective elimination is greatly exaggerated.

In conclusion, it appears that neither genetic research nor studies upon elimination closely limit the possibility that selection has played a very important part in evolution. In addition, recent field-studies[6] demonstrate novel facts of common occurrence which must apparently be ascribed to the action of this factor. Hence as was suggested in the beginning, Dr. Pearl would seem to over emphasize the importance of variation, and to attach too little significance to selective agencies in determining the course of racial history.

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[6] Longley, W. H., 1914, Report upon color of fishes of the Tortugas Reefs, Carnegie Inst. of Washington, Year Book No. 13, pp. 207–208; 1915, "Coloration of Tropical Reef Fishes," Carnegie Inst. of Washington, Year Book No. 14, pp. 208–209; 1916; "The Significance of the Colors of Tropical Reef Fishes," Carnegie Inst. of Washington, Year Book No. 15, pp. 209–212; 1916, "Observations upon Tropical Fishes and Inferences from their Adaptive Coloration," *Proc. Nat. Acad. Sciences,* Vol. 2, pp. 733–737.

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STUDIES UPON THE BIOLOGICAL SIGNIFICANCE OF ANIMAL COLORATION

II. A REVISED WORKING HYPOTHESIS OF MIMICRY

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ALTHOUGH zoologists know that detailed resemblance in outward appearance may occur between different species of insects which are not closely related, they do not agree in their interpretation of the facts they observe. Present knowledge, indeed, justifies nothing more than tentative explanations of mimicry; but, in this matter, observations recently reported[1] limit one's freedom of choice, since they appear to bear directly upon the validity of current hypotheses reviewed in the following pages.

The first attempt to interpret mimetic resemblance as a result of natural selection was made by H. W. Bates,[2] who writes:

> What advantage the Heliconidæ possess to make them so flourishing a group, and consequently the objects of so much mimetic resemblance, it is not easy to discover. . . . It is probable that they are unpalatable to insect enemies. . . . They have all a peculiar smell. I never saw flocks of the slow flying Heliconidæ in the woods persecuted by birds or dragon flies, to which they would have been an easy prey;

[1] Longley, "The Colors and Color Changes of West Indian Reef-fishes," *Jour. Exp. Zool.*, 1917. It happens that the present paper will appear slightly before that cited.

[2] *Trans. Linn. Soc. Lond.*, Vol. 23, p. 510.

nor, when at rest on leaves, did they appear to be molested by lizards or the predaceous flies of the family Asilidæ, which were very often seen pouncing on butterflies of other families. If they owe their flourishing existence to this cause, it would be intelligible why the Leptalidæ, whose scanty number of individuals reveals a less protected condition, should be disguised in their dress, and thus share their immunity.

Bates himself points out the fact that "some of the mutual resemblances of the Heliconidæ seem not to be due to the adaptation of the one to the other, but rather, as they have a real affinity, . . . to the similar adaptation of all to the same local, probably inorganic conditions." Thus the application of his hypothesis was limited from the beginning.

Fritz Müller[3] showed how those instances of resemblance which his predecessor ascribed to the influence of Lamarckian factors might be aligned with the Darwinian hypothesis. In Meldola's[4] translation of his original paper his idea is expressed as follows:

> What benefit can one species derive from resembling another, if each is protected by distastefulness? Obviously none at all, if insectivorous birds, lizards, etc., have acquired by inheritance a knowledge of the species which are tasteful or distasteful to them—if an unconscious intelligence tells them what they can safely devour and what they must avoid. But if each single bird has to learn this distinction by experience, a certain number of distasteful butterflies must also fall victims to the inexperience of the young enemies. Now if two distasteful species are sufficiently alike to be mistaken for one another, the experience acquired at the expense of one will likewise benefit the other; both species together will only have to contribute the same number of victims which each of them would have to furnish if they were different.

Recent years have been marked by a tendency upon the part of some observers[5] to extend the bounds of the Müllerian associations, until in a given fauna a large proportion of the insects which show the same color combination are included in one bionomic group. Scores of species have, indeed, been assigned to some, and it has

[3] *Kosmos*, May, 1879, p. 100.

[4] *Proc. Ent. Soc. Lond.*, 1879, p. xxvii.

[5] Marshall and Poulton, "The Bionomics of South African Insects," *Trans. Ent. Soc. Lond.*, Vol. 41, pp. 287–584.

been alleged that very many types from the same region show the influence of one or another of the dominant Müllerian aggregations. Müller's hypothesis, however, has not attained preeminence solely by extension to newly discovered cases of resemblance, but has prospered in many instances at the direct expense of the Batesian conception. Its changed fortune depends largely upon Dixey's[6] discovery of mimetic attraction, or reciprocal mimicry.

This relation, which it is held exists between many insects previously considered typical Batesian couples, suggests that the observed resemblance involves mutual adjustment. But the idea that each species introduces into its own pattern elements characterizing that of the other, and thus contributes actively to the development of a common type of coloration, is intelligible in terms of the mimicry hypotheses, only if the superficial resemblance so attained is mutually advantageous. Whatever justifies change in the interpretation of fact lies here; for, if it be assumed in the beginning that the likeness noted must have arisen either through the action of Batesian or Müllerian factors, it must be admitted that the notion of mutual advantage seems more fitly associated with the latter: two species, each of which enjoys marked immunity, seem better able to force reciprocal concessions in their achievement of resemblance, than they should, if there were great disparity in their means of defence.

Perhaps the most obvious suggestion from Dixey's research is not, after all, that a closer approximation to truth may possibly be attained through reclassification of mimetic resemblances, but that an intergrading series of alleged Batesian and Müllerian mimics is perfectly conceivable, in which no known test could possibly determine the occurrence of a natural break. Hence there would seem to be only historical reasons for maintaining the two categories.

It is noteworthy that neither hypothesis to which reference has been made was in its original form an attempt

[6] *Trans. Ent. Soc. Lond.*, 1894, pp. 249–334.

to explain the existence of conspicuous creatures. The sole concern of each was the interpretation of resemblances, which were later commonly considered mere incidents in the attainment and use of warning colors. This shifting of emphasis from "likeness which is perfectly staggering"[7] to conspicuousness, which failed to elicit a single outspoken comment in the original papers of Bates and Müller, is distinctly chargeable to Wallace,[8] who extended his hypothesis of the functional conspicuousness of bright colors, until it included the facts of mimicry.

Abbott H. Thayer[9] has proposed an explanation of the resemblance between unrelated species of butterflies, which is consistent with his thesis that the foremost function of animal coloration is concealment. This hypothesis is Darwinian in principle, but in practise is directly opposed to current selectionist opinion. It is purely speculative, and is stated as follows:

> It is surely conceivable that in a certain region, one particular form of flower-scenery representation may furnish such advantage to butterflies as to cause many widely separated species to become modified till they wear a common aspect, and it is conceivable also that there would be one common form of wing that would best lend itself to this scheme.

More recently Thayer[10] adds that in the paper from which the excerpt above is taken he ascribed more importance to butterflies' resemblance to flowers, as compared to their rendering of scenery, than he should at the later date; but this necessitates no modification of his idea that mimicry is mere incidental resemblance between species, which, through selection based upon the obliterative effect of their coloration, conform ever more closely to one ideal representation of their common background.

If the Darwinian ranks are divided upon the question of the prevalence of conspicuous types of coloration, similar though less open dissension appears among their op-

[7] Bates, *l. c.*, p. 507.

[8] Darwinism, p. 239. Macmillan and Co., 1891.

[9] *Trans. Ent. Soc. Lond.*, Vol. 42, p. 557.

[10] See Thayer, Gerald H., "Concealing Coloration in the Animal Kingdom," Appendix *B*, p. 251. Macmillan and Co., 1909.

ponents. To Piepers,[11] for example, specific coloration is in large part a visible token of internal organization determined from time immemorial, a result of orthogenetic evolution capable, however, of being accelerated or retarded by external conditions. To Packard[12] it represented a racial response of organism to environment, and mimicry seemed an effect of exposure to conditions of the same kind. But if evolutionary processes be largely beyond the control of external agents, and species spring from species through internal reorganization, as one configuration follows another in the kaleidoscope, one should anticipate that many color combinations of exaggerated conspicuousness might result. If, upon the other hand, the development of color and pattern be determined by animals' environment, their coloration may well repeat dominant notes from their surroundings. Hence it is not surprising to find that Packard expresses hearty appreciation of Thayer's discoveries, and Piepers, despite his anti-Darwinian attitude, might have much in common with Wallace and Poulton. Thus the full series of contradictions is rounded out, and the unsettled state of opinion concerning mimicry, or indeed the whole matter of animal coloration, is apparent, for the two qualities, utility and conspicuousness, openly or tacitly affirmed or denied, may be ascribed in every possible combination to the color and pattern of a single organism.

It may apparently be stated safely without qualification that the bright colors of tropical fishes as a class are correlated with the animals' habits, and, in the case of all but red, distinctly repeat tones characterizing their normal environment. But, other things being equal, no one will maintain that any system of external pigmentation could be less conspicuous than one conforming to this principle. Therefore, among these creatures at least, the occurrence of bright colors in contrastive patterns is not inconsistent with the idea that the forms that display them are as inconspicuous as may be under the conditions in which they

[11] "Mimikry, Selektion und Darwinismus." Leiden, 1903.
[12] *Proc. Amer. Philos. Soc.*, Vol. 43, p. 421.

live. It is this fact that necessitates a review of hypotheses of animal coloration that postulate conspicuousness; for one cannot safely disregard the suggestion that principles applicable to one group of animals may be valid also in the case of others.

It is of interest to note that Chapman[13] studied the birds of Trinidad under favorable conditions and observed that distinct types of coloration marked those of different habits. The most brilliant species occupy the most exposed positions in the treetops. More sedentary forms inhabiting the body of the trees are largely green, and brown predominates in the coloring of those that climb upon the tree-trunks, frequent the undergrowth near the forest border, or live upon the forest bottom.

These ecological records are a mere incident, a by-product of their author's activity. Through lack of detail they possess no great intrinsic value, but are highly significant in their present setting. Mention should also be made of Potts'[14] observation that shrimps living symbiotically with crinoids upon the Australian reefs repeat the colors of the forms with which they are associated. That comparable facts regarding other groups of animals are not available is immaterial. If the bright colors of tropical birds, fishes and some crustacea repeat those of the animals' respective environments and minister to the inconspicuousness of their possessors, it is of interest to inquire what data and reasoning support the contention that in insects similar combinations bear a different relation to the colors about them and discharge another function.

It appears first, from the statements of a number of their more prominent advocates, that there are fundamental theoretical objections to the hypotheses of warning coloration and mimicry.

Poulton[15] remarks that the acquisition of an unpleasant taste or smell, together with a conspicuous appear-

[13] *Bull. Amer. Mus. Nat. Hist.*, Vol. 6, pp. 19–20.
[14] Carn. Inst. Wash., *Papers Dept. Mar. Biol.*, Vol. 9, pp. 71–96.
[15] *Proc. Zool. Soc. Lond.*, 1887, p. 192.

ance, is so simple a form of protection, and yet *ex hypothesi* so absolutely complete, that it seems remarkable that more species have not availed themselves of this mode of defence. He argues that if once their potential vertebrate enemies were driven to eat any such insects in spite of their unpleasant taste, they would almost certainly soon acquire a relish for what was previously disagreeable, and the insects would be in great danger of extermination, having in the meantime become conspicuous by gaining warning colors. He concludes that if this reasoning is correct, it is clear that this mode of defence is not necessarily perfect, and that it depends for its apparently complete success upon the existence of relatively abundant palatable forms: in other words, its employment must be strictly limited.

Dixey[16] encounters the same difficulty in his consideration of Batesian mimicry. He observes that in this relation the advantage is all on the side of the edible mimicking species, whose existence is, indeed, a source of danger to the form mimicked, inasmuch as any experience gained by tasting the former would be used to the detriment of the latter. From these considerations he believes that such an association can exist only when the numbers of the one species are insignificant in comparison with those of the other. Upon this point he is in essential agreement with Wallace,[17] who states that mimicry has been shown to be useful only to those species and groups that are rare and probably dying out, and would cease to have any effect should the proportionate abundance of mimic and model be reversed.

Here, then, are two hypothetical types of association whose persistence admittedly depends upon the maintenance of definite though undetermined ratios between their components. Hence it devolves upon those who hold that the assumed relations are real, to outline some system by which the due proportion of protected and unpro-

[16] *Trans. Ent. Soc. Lond.*, 1897, pp. 317–332.
[17] *Westminster Review*, Vol. 32, N. S., p. 35.

tected forms might be maintained through the interaction of natural factors.

In an attempt to avoid this preliminary difficulty Poulton declares that it has always been recognized that an insect may be distasteful to one vertebrate enemy, but palatable to another. He suggests a different counterbalancing limit, which he admits would certainly in time become identical with the other. He argues that a vertebrate enemy may be forced by stress of hunger to eat an unpalatable insect, by implication asserts that this adaptability of potential enemies of forms tending to assume warning colors would limit the number of species developing them, and considers the truth of his suggestion confirmed by experimental tests.

It seems, however, that as long as warning coloration confers any advantage, other species should move in the same direction, for the same reasons that those did in which it first appeared. The number of warningly colored species should therefore increase until the situation conceived by Poulton materialized. But in that event the experimental proof that hungry animals are not so fastidious as those that are well fed is insufficient to meet the exigencies of the situation; for one has no reason to believe that animals which might become adjusted to the new condition would confine themselves to a diet of insects whose warning coloration was recently acquired, and would leave intact the vested interests of those that first attained it. Indeed, if one is permitted to speculate, it seems not wholly unreasonable to anticipate a swing of the pendulum in the other direction, so that conspicuous forms might face the possibility of almost complete extinction. This is suggested by the well-known fact that interspecific adjustments are not rigid, and that a state of approximate equilibrium is frequently modified by climatic or other factors, and restored only after a series of more or less definite oscillations. Examples in point are furnished by Howard's[18] observations upon ichneu-

[18] "New Nature Library," Vol. 7, Pt. 1 (The Insect Book), p. 68. New York, Doubleday, Page and Co.

mon flies and their primary and secondary parasites, and more particularly by those of Forbes[19] and Bryant[20] upon the feeding habits of birds.

In his paper, which has been cited above, Dixey was not discussing the validity of the mimicry hypotheses and has not attempted to explain what limits the numbers of a mimic in proportion to those of its model. Wallace also failed to elucidate the mystery, leaving his readers to find the way out of their difficulty as they might best be able. Search elsewhere for helpful suggestion in the matter yields little of value. Poulton[21] holds that ichneumon flies are particular enemies of the larvæ of "protected" Lepidoptera, but this idea need not be taken very seriously at present, for it is apparently based upon the observation of a high proportion of parasitized individuals among such forms rather than upon comparative statistics covering "unprotected" species as well. Even if it were true, while it would bolster the warning color hypothesis, it would aggravate the situation regarding Batesian mimicry. Therefore only one conclusion is possible: The theoretical objections they themselves have raised are not adequately met by spokesmen for the two hypotheses.

What observed facts support the contention that many animals are made conspicuous and profit by the striking combinations of color they display, should next be determined.

In 1867 Wallace[22] suggested before the Entomological Society of London that, as a rule, brilliantly colored larvæ are distasteful to birds, expressed his desire for information and his gratification, if any who kept birds, particularly indigenous species, would make experiments with different larvæ to ascertain which were eaten and which rejected. Members of the society and others have repeatedly acted upon his suggestion and commonly

[19] "The Regulative Action of Birds upon Insect Oscillation," *Ill. State Lab. Nat. Hist.*, Bull. No. 6.

[20] "The Condor," Vol. 13, 1911, pp. 195–208.

[21] "The Colors of Animals," p. 182. New York, D. Appleton and Co., 1892. For additional data, see *Trans. Ent. Soc. Lond.*, Vol. 41, p. 337.

[22] *Proc. Ent. Soc. Lond.*, 1867, pp. lxxx–lxxxi.

agree that the results of the experiments support his hypothesis, whose application has meanwhile been greatly extended. But the spirit in which the observed facts are interpreted precludes all possibility of the main inference drawn from them being seriously considered by any unprejudiced critic who examines the argument.

If a dull-colored insect be eaten, this is held by implication to lend strong support to the hypothesis of warning coloration; for palatable insects must be inconspicuous or be destroyed. If, upon the contrary, such an insect be rejected, its distastefulness may be a useless character, an accident of metabolism, or vestigial, or may be related to functional distastefulness in preceding stages of the life history; in any case "it must be remembered that an unpleasant attribute must always appear in advance of the warning coloring,"[23] so the result is not inconsistent with Wallace's suggestion. Again, if a bright-colored insect be rejected, this accords with his contention; and finally, if such a one be accepted, experimenters are prone to agree with J. Jenner Weir[24] who writes:

> But I am by no means inclined to attach undue importance to this fact, because the birds, being in a state of confinement, might readily be expected to eat insects, which in a state of nature, with a less limited choice, they would reject.

It is unnecessary to review these experiments in detail and to attempt to evaluate them, for this has already been done thoroughly by McAtee,[25] who shows their utter futility by comparing typical observations upon caged birds with the facts revealed by analysis of the stomach contents of wild specimens of the species experimented upon. Regarding information from such sources as that last-mentioned he writes:

Since this evidence is sufficient in itself and since experimental data must be supported by it, why perform the experiments? The same time spent in collecting trustworthy data regarding the natural food

[23] Poulton, "The Colors of Animals," p. 176.
[24] *Trans. Ent. Soc. Lond.*, Vol. 7, Ser. 3, p. 22.
[25] *Proc. Acad. Nat. Sci. Phila.*, 1912, pp. 281–364.

habits of animals would bring much greater returns, and the result would be truth, not imaginative inferences from abnormal behavior.

Before concluding the discussion of this matter it should be stated that even if it were proved that bright-colored insects are distasteful, it might not be inferred fairly that they are conspicuous, or that their coloration has a specific warning (aposematic) function. Feeding experiments under ideal conditions might determine the presence or absence of distastefulness, and show to what extent it is correlated with the display of color combinations of a particular sort. But even a high degree of correlation between unpalatability and gaudy coloration proves that the latter is conspicuous, no more than the demonstration that an unknown substance has the approximate *hardness* of gold proves that it has the same *specific gravity;* for brightness, or vividness of coloration, and conspicuousness are incommensurable.

Except in Thayer's contributions, confusion upon this point has prevailed from the beginning, when Wallace used interchangeably the expressions, "brilliantly colored larvæ" and "caterpillars conspicuous by their lively coloration." But the assumption that some animals are conspicuous, or, in other words, that while their habits remain the same their average visibility might be greatly diminished by another system of coloration than that they possess, can neither be adequately defended nor refuted, until the results of such exhaustive studies of animals' habits as have rarely been attempted are available. The distribution of animals must be studied intensively, for the division of the world into provinces and the subdivision of these into their major components by gross dissection is not a technique of sufficient refinement to discover the essential relations between organism and environment.

Passing to another phase of the matter, the value of recorded observations upon the conspicuousness of insects may be shown very clearly.

Bates saw no Heliconidæ attacked by dragonflies or

other predaceous insects which often pounced upon butterflies of other families. But Poulton[26] finds, to state it mildly, that "there is good reason for believing that such attacks are not rarely made, and that predaceous insects are important enemies of aposematic butterflies." He also writes[27] of the Batesian hypothesis:

> This was not, as has been generally supposed, originated by Bates during his years of observation in the Valley of the Amazon. It arose in his mind after his return home, when he came to examine his collection and to reflect upon his experiences.

Under these circumstances the uncorroborated testimony of this witness concerning matters which are not known to have been carefully investigated in the Brazilian wilderness and are not determinable from the study of preserved material is of little immediate consequence. Among subjects regarding which his opinion can at present he held only in slight esteem, and concerning which he expressed himself in other communications than his original paper upon mimicry, the inherent conspicuousness of bright colors may be justly included.

The positive assertions in the following quotation describing conditions observed by an entomologist in British Guiana are also instructive; the same is true of their author's naïve conclusion.

W. J. Kaye[28] writes:

> The forest is dark and gloomy, and throughout the greater part of the year excessively damp owing to a superabundant rainfall. The character of the vegetation is always the same, as even in the dry season the trees are never otherwise than a fresh green. It is not surprising, therefore, that practically the whole of the Lepidoptera, excepting, of course, the several species of *Morpho,* present a very uniform, somber tone of coloration. Even the very fine and brightly colored *Heliconius catharinæ, H. astydamia* and *H. egeria* do not strike one in their surroundings as being particularly gaudy, and one is bound largely to admit the assertion of Abbott H. Thayer that many species we call conspicuous are not really so in their natural surroundings. It must, however, have been quite impossible for nature to have evolved such

[26] *Trans. Ent. Soc. Lond.,* Vol. 41, p. 328. See also Vol. 44, pp. 323–409.
[27] "Essays on Evolution," p. 211. Oxford, Clarendon press, 1908.
[28] *Trans. Ent. Soc. Lond.,* Vol. 44, p. 412.

minutely close resemblance in unrelated groups without the aid of Müllerian mimicry.

It remains to state that among Lepidoptera different species have their characteristic attitudes of rest, frequent different places, fly at different levels, and are active at different times in the day. Even the two sexes of some species, and these are commonly dimorphic forms, do not haunt the same stations. It is certainly a pregnant fact, which we may accept since Wallace[29] gives independent testimony to the same effect, that Bates[30] observed many, apparently scores, of species, in which, as he says, the sun-loving males flaunted their gaudy hues in open places, while their respective females, soberly clad, frequented the forest shades.

These facts and others that might be cited are indications of diversity of habit among insects comparable with that which among fishes is correlated with the display of different types of inconspicuous coloration. They suggest that in this group as well, external pigments are distributed among species according to an intelligible system other than that whose existence is commonly inferred. But if this should eventually prove to be true, we must have an explanation of mimicry without appeal to the concept of warning colors.

Such a hypothesis has in fact been formulated by Punnett,[31] for in spite of his apparent belief in the conspicuousness of many species of butterflies it happens that he lays no stress upon it in his consideration of the origin of mimetic resemblance. His hypothesis, as he fully recognizes, is at present little more than naked suggestion. It is ingenious, is stated attractively in the current idiom of genetics, and is effectively displayed against a background of destructive criticism of its predecessors, from which it differs in minimizing the influence of nat-

[29] *Trans. Ent. Soc. Lond.*, Vol. 2, Ser. 2, pp. 253–264.

[30] "The Naturalist on the River Amazon," p. 291. Reprinted, New York, D. Appleton and Co., 1892.

[31] "Mimicry in Butterflies." Cambridge, University Press, 1915.

ural selection. It proposes an entirely new explanation of mimicry in the following terms:

> If we assume that sudden and readily appreciable variations of the nature of "sports" turn up from time to time, and if these variations happen to resemble a form protected by distastefulness so closely that the two can be confused by an enemy which has learned to avoid the latter, then there would appear to be good grounds for the mimicking sport becoming established as the type form of the species. . . . On this view natural selection in the form of the discriminating enemy will have played its part, but now with a difference. Instead of building up a mimetic likeness bit by bit it will merely have conserved and rendered numerically preponderant a likeness which had turned up quite independently. . . . Why variations on the part of one species should bear a strong resemblance to other, and often distantly related, species is another question. . . . The occurrence of mimetic resemblances is the expression of the fact that color pattern is dependent upon definite hereditary factors of which the total number is by no means very great. As many of the factors are common to various groups of butterflies, it is to be expected that certain of the color patterns exhibited by one group should be paralleled by certain of those found in another.

Upon examination these statements appear to embody a formal explanation of the facts to which it is difficult to take exception. Hence it must be admitted that this is perhaps the goal toward which with regard to this problem naturalists have been working for more than half a century. But while his hypothesis may be correct, its author's reasons for deeming it so seem quite insufficient.

The chief points of support on which it rests are the following, which are not arranged in the order of importance assigned to them:

1. The difficulty of finding the appropriate enemy which shall exercise the discrimination postulated by current hypotheses.

2. The alleged fact, mathematically demonstrated, that reciprocal mimicry between two species can not be established by selection of a long series of slight variations.

3. The theoretical difficulty of the initial variation in cases other than that above, for it seems reasonable that if the ancestral types from which mimic and model are derived were in the beginning very unlike in appearance

no slight departure from one in the direction of the other could have selective value.

4. The non-appearance of intermediates when a form which is assumed to have been derived from another by selection of a series of heritable variations (mutations) is crossed back with the original.

5. The apparent fact that the three females of the polymorphic oriental butterfly, *Papilio polytes*, occur in proportions which are approximately the same now as fifty, or possibly one hundred and fifty years ago, although it may be demonstrated mathematically that if either of the two mimetic forms possesses even a slight advantage over that from which they are assumed to be descended, this should have appeared in an altered ratio while they continue to live and breed together under the same conditions.

6. The fact that certain variations induced by differences in temperature or humidity are not directly inherited, since it is alleged that this limits the material upon which selection might be supposed to operate.

Of these points the first three may be more conveniently considered later with other common objections to current hypotheses of mimicry. The remaining three will be next examined in order.

Punnett accepts Fryer's[32] conclusion that in *P. polytes* the two supernumerary females which resemble *P. aristolochiæ* and *P. hector* differ genetically from the third female (which resembles the male) to the extent of one and two Mendelian factors, respectively. He cites the fact that there are forms of sweet peas, for example, which are known to have arisen as sudden sports, and behave in heredity as though they differed from the normal by a single factor. Hence he infers from analogy, first, that the two mimetic butterflies sprang from the primitive type by one or two mutations, as the case may be, and, as a corollary, that resemblance to their models was not attained by gradual shaping of their destinies

[32] *Phil. Trans. Roy. Soc. Lond.*, Vol. 204 *B*.

through the accumulation of lesser variations by natural selection.

But Castle[33] still contends vigorously that a single genetic character may undergo quantitative change under selection, and if this be true the difference finally attained by two forms differing only in one factor might follow from the accumulation of an indefinite number of slight variations. Still the complete abandonment of Castle's position would not save Punnett's argument. For if reported observations and inferences concerning *Drosophila*[34] are correct, a red-eyed strain has given rise to white by mutation and this in turn to eosin, which being crossed with the original red gave in the F_2 generation offspring in the proportion of three red to one eosin.

It is quite immaterial whether one explains these relations upon the hypothesis of multiple allelomorphs, or, as Punnett[35] prefers, upon the assumption of complete coupling of factors. In the former case one must admit that by breeding experiments the end product of a series of mutations can not be indubitably distinguished from one that results from a single modification of the affected factor. In the latter, one must grant that the occurrence of the grandparental types in the offspring of hybrid parents in the ratio of three to one is no proof that the grandparents themselves differed in respect to one character alone, or that the difference between the two resulted from changes occurring at one time in the germinal constitution of an ancestor of one of them. Hence, in so far as this argument is concerned, there is in the case of *P. polytes*, for example, no assured reason for supposing that the *aristolochia*-like form did not attain its present appearance by a series of steps, of which a number of the later at least were preserved by virtue of the advantage they conferred upon the individuals in which they appeared.

[33] "Genetics and Eugenics," p. 188. Cambridge, Harvard University Press, 1916.

[34] Morgan, Sturtevant, Müller and Bridges, "The Mechanism of Mendelian Heredity," p. 164. New York, Henry Holt and Co., 1915.

[35] *Jour. of Genetics*, Vol. 5, pp. 37–50.

Little need be said regarding the inference that the constancy of the ratio in which the females of *P. polytes* seem to have occurred for many years shows that natural selection does not exist for this species in Ceylon, or else that its force is so slight that in half a century, and perhaps in a century and a half, it has produced no effect appreciable to the method of examination employed. This is valid only if it is true as postulated that the various types of *P. polytes* constitute "a population living and breeding together under the same conditions." But it is gravely to be doubted that this indispensable condition is fulfilled. We have some evidence (that of Bates and Wallace already cited) that butterflies which differ in color differ in habit, and if it should appear that the colors of butterflies in general are correlated with and repeat those of their surroundings, Punnett's fifth point is forever invalid. For it will be impossible to establish by observation the universal negative that is required, which is, of course, that the three types of female do not differ in any constant respect in their normal behavior.

Regarding the sixth point, which has reference chiefly to the fact of seasonal dimorphism among butterflies, it must first be affirmed that although the induced changes differentiating the broods of the spring and summer, or wet and dry seasons, are not directly inherited, the capability of responding definitely to the physical stimulus of changed temperature or humidity is a heritable racial trait.[36] The seasonal variations in the coloration of butterflies may be analogous upon the whole with the instantaneous color changes of tropical fishes, which also occur in response to external stimuli. The latter, however, follow more quickly than the former upon appropriate stimulation; they are reversible; and are known to be normally adaptive, since they reduce the conspicuousness of the individuals in which they appear. It may eventually prove to be a fact that instantaneous adaptive color adjustments, the phenomena of seasonal and sexual dimor-

[36] Professor Gerould first called attention to this fact in THE AMER. NAT., Vol. 50 (1916), pp. 310–316.

phism, and polymorphism all have the same biological significance, *i. e.*, that they represent different ways in which the coloration of a species exercises its obliterative function in a greater variety of circumstances than would be possible if it were uniform. Upon this view of the matter there would seem to be no reason why color variations of the seasonal sort should not provide material for evolution by natural selection.

Before suggesting another possible explanation of the fact of mimetic resemblance it seems desirable to state more specifically why certain of those already mentioned seem improbable.

Some color patterns are apparently limited to fishes whose habits are similar. Others occur which have survived the introduction of marked structural changes and are now the common property of whole families or groups of families, whose manner of living varies decidedly from species to species. There is one such system of coloring among grunts, groupers and snappers (Hæmulidæ, Epinephelinæ, and Lutianidæ), and Labrids and Scarids share another. In each pattern modifications may be noted which seem particularly appropriate under the local conditions in which they appear. Individual elements may lose all semblance of the original, and yet the nature of the whole be not obscured. But these facts make one skeptical regarding Thayer's hypothesis, for if, in butterflies too, detail is less important than the appropriate effect of the whole, the probability is remote that for different environments complex, ideal, protective or concealing patterns exist, whose slightest spot is so significant that there is marked tendency for forms of different racial endowment to attain them, if their habits are similar.

The same facts militate against such a conception as Packard's, that mimicry is a result of similar reaction to the direct influence of one set of external conditions. For coloration is characterized by such conservatism or inertia, and the same elements of pattern appear in such a variety of habitats, that the power of environmental in-

fluence to induce uniformity of coloration seems discredited.

If the possibility of the direct influence of local climatic factors be excluded, Piepers' hypothesis, that mimicry is largely due to species having independently attained the same stage of development orthogenetically, leaves the facts of geographical distribution of mimics and their models enshrouded in mystery. That this is a very real difficulty follows from Punnett's statement,[37]

> Examples of close resemblance between butterflies which live in different parts of the world are relatively rare and serve to emphasize the fact that the great bulk of these resemblance cases are associated in pairs or little groups.

Finally, instances of mimicry are, after all, only selected examples of resemblance, and it is desirable, if possible, to formulate an explanation that will apply to all equally well. But whether the likeness between them rises by sporting or otherwise, it is not to be supposed that Phasmids and green leaves or dry twigs possess Mendelian factors in common. Therefore it seems profitable to proceed for a little upon the assumption that mimicry is in some cases at least a visible token of the fact that the species manifesting it are linked by some bond other than common descent, common habit, or exposure to the influence of a common environment. What this may be, does not appear, unless through mutual resemblance advantage accrues to some or all of the forms concerned.

Evidence compiled by Marshall[38] shows that birds undoubtedly attack butterflies, but others deny that they feed upon the insects freely enough to affect the evolution of their coloration, and more particularly the mimetic resemblances between different species. Hence there is plainly a question at issue concerning the sufficiency of an assigned cause to produce a stated effect. Under the circumstances any evidence tending to show that the frequency of birds' attacks has been underestimated, or that

[37] *L. c.*, p. 54.

[38] *Trans. Ent. Soc. Lond.*, 1909, pp. 329–383.

their influence may be supplemented by that of other enemies is of the greatest interest.

In this connection Swynnerton's[39] observation that of twenty small bird excreta collected in the African forest no less than eighteen contained scales and small wing fragments of Lepidoptera has suggestive value. But, for the moment at least, it is more important that it appears that mimicry might be initiated and advanced by indiscriminate feeders, including lizards and insectivorous insects, provided only that they possess color vision. For to whatever extent such influence prevails it obviates the necessity of appeal to the effects of discriminate feeding by birds or other animals, and makes it possible to forestall the criticism to which reference has been made above.

Therefore it is suggested as a tentative explanation of mimicry, that it has commonly arisen as a result of bionomic pressure applied first by discriminate or indiscriminate feeders, which by elimination of unadapted variants have forced their accustomed prey to assume color combinations which most effectually conceal it in its normal environment. In addition, for no demonstrated reason, in a few of the many thousands of cases in which colors adapted to the environment and habits of their possessor have been evolved, patterns have appeared which have been sufficiently like one another to deceive enemies which exercise discrimination in their choice of food. Beyond this point the evolution of resemblance may have proceeded according to accepted formulæ, but without conspicuousness being involved at any point in the process.

It is submitted that in our present state of ignorance this construction may be placed upon observed facts rationally and without exposure to the criticism that has been directed against other attempted interpretations. However, the chief classes of facts to be explained and the most serious objections registered against the Neo-Darwinian hypotheses of mimicry will be presented, that

[39] *Ibis*, 1912.

the reader may judge whether a passage between Scylla and Charybdis may be made in safety.

Professor Poulton's extensive studies have convinced him that the evolution of mimetic resemblance has been directed by natural selection,[40] yet the evidence upon which his conclusion rests may be taken over bodily and supports the revised hypothesis as consistently as that to whose service it was originally dedicated. There is nothing anomalous in finding mimic and model living under the same conditions, certain groups of insects showing the same series of local color varieties, or such diversity of coloration appearing in one group of butterflies or moths as allies them outwardly with different "protected" genera. The same is true of the fact that insects with every variety of larval experience as adults possess the same type of coloration, that mimetic females are more common than males, or that the common coloration possessed by mimic and model is attained in the most diverse fashion, that is, that cases of mimicry are typical instances of analogy. Throughout the whole series of observations the points of agreement and difference are consistent, as far as is known, with the fundamental assumption that color and habit are associated variables.

Passing to the negative side of the argument, we may first consider the statement that it is impossible that reciprocal mimicry should have been brought about by natural selection of small variations. Punnett has this idea from Marshall[41] and uses it to emphasize the difficulty of the initial variation even in cases where it might seem that the theoretical advantage to be gained from mutual resemblance by two species would simplify the attainment of likeness. But Dixey,[42] against whose position the argument was originally directed, has exposed its unsoundness by calling attention to a number of critical

[40] See "Natural Selection the Cause of Mimetic Resemblance and Common Warning Colors" in "Essays on Evolution," 1908.

[41] *Trans. Ent. Soc. Lond.*, Vol. 45, pp. 93–142.

[42] *Trans. Ent. Soc. Lond.*, Vol. 45, pp. 559–583.

points which his opponent had failed to take into consideration.

It may be added that Marshall's reasoning rests upon what is without much doubt a baseless assumption, for he follows Müller in postulating that two species of distasteful insects will lose the same absolute number of individuals through attacks of ignorant enemies which in the beginning recognize neither of them. As a matter of fact, if two species differing in no respect except appearance are represented in the same area by 100,000 and 5,000 individuals, respectively, as Marshall assumes, the chances are 20:1 that any animal making an independent test of the food resources of its environment would first meet the more abundant form. Unless it learns its lesson perfectly from a single experience, the chances are essentially the same that it will kill another butterfly of the same kind before it encounters one of the second distasteful sort. But if most inexperienced enemies learn at the expense of one species that *some butterflies are not edible*, it is scarcely to be supposed that they will undergo as many unpleasant experiences before they retain an impression of the disagreeable character of the other. Hence Marshall's criticism can not be considered at present a valid objection.

Packard[43] believed that the concept of Müllerian mimicry had been overextended. He thought that in accumulating so many examples of warning coloration in their "Bionomics of South African Insects" Marshall and Poulton[44] in particular attempted to prove too much. Why an association of some scores of species representing many orders of Mashonaland insects should be pivoted upon the bitter-flavored beetle, *Lycus*, though some members of the group seemed more amply protected from attack by birds and lizards, was not clear. Yet one dares not be dogmatic in such matters, for the wasp, *Pompilus*, though more adequately equipped for defense than any other member of the association, may have drifted toward

[43] *Proc. Amer. Philos. Soc.*, Vol. 43, p. 424.

[44] *Trans. Ent. Soc. Lond.*, Vol. 41.

it at a comparatively late date, when the relatively slight distastefulness of a large number of insects of one type of coloration subtended a larger angle in the consciousness of insectivorous animals than the greater unpalatability of any single form. However, the idea that what has been considered mimicry is too common, and that in general the most effectively protected types should be the nuclei of the Müllerian combinations, is certainly not wholly unreasonable.

One of the chief reasons for believing in the existence of warning colors, and particularly of common warning colors, is the fact that some families of insects have slight range of color and pattern compared with others. Mayer[45] found that "the 200 species of *Papilio* in South America display 36 distinct colors, while the 450 species of Danaoid Heliconidæ exhibit only 15," and that "there is no lack of individual variability among the species of the latter, yet as a whole they vary but little from the two great types of color-pattern represented by *Melinæa* and *Ithomia*." To explain these facts he felt obliged to resort to Müller's hypothesis, but if instead of thinking of Ithomiinæ and Papilionidæ one considers Holocentridæ and Labridæ, an alternative solution appears. The squirrel fishes seem to be of red or reddish coloration the world over, but their habits are equally invariable, while the Labrids' diversity of coloring is no greater than that prevailing in the varied environments in which they live.

Such facts indicate the necessity of making detailed studies of the coloration of tropical Lepidoptera and correlating the facts discovered with the insects' distribution and behavior. When this is done there is reason to suppose that combinations of the same colors will be found upon animals of the same habit, which would have been as they are in many species, if any or all the others which display the same combinations had never existed. That is to say, it is probable that much that has masqueraded as Müllerian mimicry is nothing but the result of con-

[45] *Bull. Mus. Comp. Zool. Harv. Coll,* Feb., 1897, p. 225.

vergent evolution, which has been difficult to explain because of the deep-seated misconception that has prevailed regarding the function of animal coloration.

Dewar and Finn[46] cite a number of instances of resemblance between mammals, and others between birds, whose ranges coincide at no point. For the most part these likenesses do not seem comparable with the clearest cases of mimicry among insects in the degree of detailed resemblance they involve, and scarcely seem to rise above the level of interesting coincidences. It is unquestionably true, nevertheless, that such degree of likeness as may spring up between two species whose bionomic association is impossible on account of differences in geographical distribution, may also arise between species of one region without reference to the action of natural selection directed toward the production of resemblance.

Lock[47] states that Syrphid flies, which closely mimic small bees and wasps whose habits are similar to their own, are surprisingly numerous in southern Japan, and that their resemblance to bees is particularly noticeable, though these are conspicuous by their absence. Hence the question arises, how the flies can benefit by their resemblance to them: to which one must apparently answer, that under the conditions stated, the bee-like disguise can, as such, be of little value. But this query is overshadowed in interest by another: If the Syrphids are unprotected and driven by their enemies to assume the appearance of defended forms, how do they survive in regions where their disguise possesses no suggestion of unpalatability.

The idea is not to be entertained for a moment that Lock would be at a loss for an answer. But if the concept of warning coloration be abandoned, there is no reason to suppose bees less perfectly adapted in color and form than other animals to their respective modes of life. Bee-like flies whose habits resemble those of bees should there-

[46] "The Making of Species," pp. 242–245. London and New York, J. Lane, 1909.

[47] "Recent Progress in the Study of Variation, Heredity and Evolution," p. 58. London, John Murray, 1907.

fore be well able to exist beyond the range of models, which they may have mimicked in other times and places, if their particular type of coloration is as well suited to the new environment as to the old.

An apparent inconsistency in the Batesian and Müllerian hypotheses as at present interpreted has been frequently noted by hostile critics. To Reighard[48] it appears, for example, that if insectivorous vertebrates have pushed the resemblance between mimics and their models to the point of apparent identity, ordinary specific differences should suffice to warn them of the unpalatability of prospective and familiar prey.

This objection is so fairly met by the revised hypothesis, and the ground for criticism so completely removed, that further comment is unnecessary. But even when no inconsistency is involved in the explanation of the facts, some will doubtless consider the resemblance of the mimic to its model, or of insects to other objects, hypertelic. It is doubtful, however, whether hypertely embodies a real difficulty. For just as two streams flowing down a tolerably smooth inclined plane of infinite length will eventually unite, if all deviations of one or both which exceed a given magnitude are blocked when they tend to increase the distance between them, so, if heritable variations in the color and pattern of a given mimic are distributed according to Quetelet's law, for example, and only the extreme forms most unlike the model be eliminated in successive generations, closer and closer resemblance between the two may appear and approach identity without appeal to that over-refinement of vision whose existence among insects' enemies is at least problematical.

It is a standing objection to the mimicry hypotheses, and indeed to the explanation of any highly complex adaptation by natural selection, that at every stage the degree of resemblance attained must have been serviceable in order to assure its survival. It is understood, however, that this objection is applicable only to stages

[48] Carn. Inst. Wash., *Papers from Tortugas Lab.*, Vol. 2, p. 315.

following the first to which the selectionist ascribes deceptive value. Resemblance resulting from undirected variation, or existing for other reasons, is not subject to this criticism.

Darwin recognized this fact and attempted to throw upon another cause than natural selection a large part of the burden of producing functional resemblance. His idea may best be expressed in his own words:

> The process of imitation probably never commenced between forms widely dissimilar in color. But, starting with species already somewhat like each other, the closest resemblance, if beneficial, could readily be gained by the above means (natural selection), and if the imitated form was subsequently and gradually modified through any agency, the imitating form would be led along the same track, and thus be altered to almost any extent, so that it might ultimately assume an appearance or coloring wholly unlike that of the other members of the family to which it belonged. There is, however, some difficulty on this head, for it is necessary to suppose in some cases that ancient members belonging to several distinct groups, before they had diverged to the present extent, accidentally resembled a member of another and protected group in sufficient degree to afford some slight protection, this having given the basis for the subsequent acquisition of the most perfect resemblance.[49]

Weismann[50] attempted to avoid the same difficulty in another way. He makes no assumption that the original difference betwen mimic and model was distinctly less than that appearing at present between typical members of their respective families, but magnifies the importance of the first slight resemblance and subsequent positive variations. He had been deceived repeatedly, at least for the moment, by similarity in the flight of different species whose colors were not the same, and held as a consequence that mere variation in the manner of flight combined with the habit of associating with the form mimicked might have prepared the way for selection.

Wallace[51] would have it that certain butterflies, having

[49] "Origin of Species," Chap. XIV.

[50] "The Evolution Theory," Vol. 1, p. 93. London, Edward Arnold, 1904.

[51] "Darwinism," p. 243.

become unpalatable through the possession of disagreeable juices, developed distinguishing marks, whether in color, form or mode of flight. He then plunges *in medias res* with the assertion that "during the early stages of this process, some of the Pieridæ, inhabiting the same district, happened to be sufficiently like some of the Heliconidæ to be occasionally mistaken for them." Thereafter, as may be anticipated, evolution proceeded merrily, and examples of Batesian mimicry were perfected in due time.

Wallace's pronouncement begs the whole question. Weismann's hypothesis is conceivably true, but lacks the support necessary to carry conviction. Darwin's idea, finally, seems to be at variance with fact, since Poulton[52] infers from his own studies that the conclusion that emerges most clearly is the entire independence of zoological affinity exhibited by mimetic resemblance.

Punnett also shows most clearly how impossible the Darwinian suggestion is, but errs when he supposes that it can not be true in many cases that model and mimic were closely alike to start with. His demonstration may be accepted that the development of mimetic resemblance has not been commonly facilitated by preexisting likeness due to racial affinity, but he has wholly disregarded the fact that the degree of likeness which it is necessary to presuppose, if mimicry has been brought about by a series of comparatively small variations, might occur for other reasons.

May we not assume,[53] for example, that the Pieridæ and Heliconidæ are usually distinctly different in their habits, and that the coloration of typical members of each

[52] *Proc. Linn. Soc. Lond.*, Vol. 26, p. 570.

[53] This can scarcely be considered a rash assumption, since Wallace states (*Trans. Ent. Soc. Lond.*, Vol. 2, Ser. 2) that the Pieridæ of the Amazon valley generally are open-ground butterflies, two genera only, *Leptalis* and *Terias*, being true denizens of the forest. He also remarks that most of the species of *Heliconia* prefer the forest shades. It is also of interest to note that he comments upon the *inconspicuousness* of some species at least of *Ithomia*, in which connection one should recall the observation of W. J. Kaye already quoted.

group is a combination of hues well suited upon the average to render them inconspicuous in such places as they commonly frequent. If this be so, the initial step toward the production of new cases of mimicry might be any one of many variations in mode of nutrition or reproduction, which would lead representatives of the first family to spend their lives after the manner of the second. Reason has already been given for believing that convergence in color would probably accompany or follow convergence in habit.

The new colors would undoubtedly appear in patterns largely determined by and reflecting the Pierian ancestry. Among fishes, as has been stated, a primitive color pattern peculiar to one or common to several closely related families is sometimes readily recognizable, in which distinct elements are apparent, now definite, now diffuse, mere stains of dyes that are not permanent. It is to be expected no less in insects that the family patterns, like finely wrought ornaments cast into the melting-pot, will be reshapen and serve new purposes. But from the welter of change and recombination which this involves may come once in many times a new grouping of characters, which suggests the pattern of another race. At this point natural selection directed toward the production of a protective design painted in colors appropriate to the environment may yield to selection working in the direction of resemblance. If so, a new pattern may be developed in the same protective colors and coupled with such change in the shape of the wings, or in other characters, as confers the additional advantage of being mistaken for a species which enjoys some measure of immunity.

Either in organization or development most animals give evidence of changes in habit much greater than the initial one herein postulated. Yet admit that these may occur, and what is already partly proved, that color is correlated with habit throughout the animal kingdom, and a theoretical difficulty that has engaged the attention of adherents and opponents of the mimicry hypotheses van-

ishes. No matter how wide the original gap between mimic and model, it may be bridged; no matter what degree of similarity between two forms may be necessary before natural selection may become effective in heightening their resemblance, it may be attained without appeal to chance that is wholly blind, for there appears to be an automatic feature in the mechanism which has hitherto escaped observation.

The ideas outlined in the preceding pages are neither a pure product of reflection nor a compromise suggested by an examination of the literature upon mimicry. They are a normal outgrowth of studies which had no preconceived relation to the problem of mimetic resemblance. They constitute a working hypothesis, and as such are submitted to those biologists, particularly entomologists, who may have opportunity to test them rigorously.

NUCLEUS AND CYTOPLASM AS VEHICLES OF HEREDITY[1]

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There have been of late several attempts to effect a compromise between theories of heredity through the cytoplasm and theories which regard the chromosomes as the vehicles of inherited characters. Conklin ('08) was the first to suggest that egg, embryonic and general phyletic characters of any stage of the organism were determined in the egg cytoplasm while the determiners in the chromosomes made their presence known only through the specific or individual adult characters. Shull (1916) has elaborated this suggestion, and has brought to its support not only the older data on maternal inheritance, matrocline hybrids and the facts of development which relate to polarity, symmetry and organ-forming substances, but has added new evidence of his own from experiments with rotifers. Most recently, Loeb, in his book "The Organism as a Whole," has advanced a similar compromise theory, based on similar evidence.

Before examining in detail the experimental basis for such a compromise, it is important that the terms to be used be clearly and unmistakably defined. The first of these is the word "determined." That a character is determined in the germ cell means that the differential, causal antecedents of that character are present in the germ cell. It does not mean that the character itself is present in the germ in any form, but rather that it is represented by substances or forces which not only *stand for* the character but in some way bring about its expression.

[1] A review made at the suggestion of Dr. H. W. Rand to whom grateful acknowledgement is due.

If this definition of "determined" is accepted, two kinds of continuity in organisms are immediately differentiated. The first sort may be called *substantial* continuity. It is the carrying over from one generation to the next of autonomous organizations of protoplasm in a manner analogous to the carrying of the bacilli of certain diseases (*e. g.*, syphilis) in the germ cell. Here the germ cell is a passive vehicle. The character is present, not determined; and its changes from fertilized egg to adult are mere proliferations. If hereditary characters were to be so viewed, and the view carried to its logical conclusion, the result would be something very like an "emboitement" theory, which facts of development have proved to be untenable. Substantial continuity is hence only a concomitant rather than a part or a method of heredity.

The second type of continuity may be called "*genetic*" continuity, and characters which are genetically continuous are those which show a new coming into being with every generation. They are developed *anew*, and their resemblance to homologous characters in the preceding generation is due to their development not from *those characters* but from homologous determinants. Characters of this type are truly determined and all hereditary characters are reducible to this type whether they are exhibited in egg, sperm, embryo or adult.

It is now possible and desirable to define the expressions "inheritance through the cytoplasm" and "inheritance through the chromosomes." The first properly means that the locus of the determiners or representatives of a character is the cytoplasm, and since it is the egg alone which contains any significant amount of cytoplasm, the expression usually means the presence of these determiners in the egg cytoplasm. "Inheritance through the chromosomes" means that the chromatic substance of the nucleus is the locus of determiners, and since the nuclear content of egg and sperm is equivalent this must also mean an equal determinative share by egg and sperm in heredity.

Are now both of these theories compatible with one definition of "determined"? Are they both possible and both necessary?

Loeb has stated the problem and the "compromise" in these words (1916, p. 245):

Question: "Is the organism nothing but a mosaic of hereditary characters determined essentially by definite elements in the chromosomes; and if this be true what makes a harmonious whole organism out of this kaleidoscopic assortment?"

Answer: ". . . the cytoplasm of the egg is the future embryo in the rough, and the factors of heredity in the sperm only act by impressing the details on the rough block."

Shull's statement is as follows (p. 6):

The cytoplasm often (perhaps usually) determines the type of cleavage, the early course of development, and in large measure the larval characters, while the adult characteristics are determined by the chromosomes.

Conklin's conclusions are (1915, p. 176):

There is no doubt that most of the differentiations of the egg cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm; but the fact remains that at the time of fertilization, the hereditary potencies of the two germ cells are not equal. All the early stages of development, including the polarity, symmetry, type of cleavage, and the pattern or relative position of future organs, being foreshadowed in the cytoplasm of the egg cell while only the differentiations of later development are influenced by the sperm. In short the egg cytoplasm fixes the general type of development and the sperm and egg nuclei supply only the details. We are vertebrates because our mothers were vertebrates and produced eggs of the vertebrate pattern—but the color of our skin, eyes and hair . . . were determined by the sperm as well as by the egg from which we came.

The same author has reiterated and somewhat elaborated the same views in an unpublished paper presented before the National Academy of Science in November, 1916.[1]

[1] Since the above was written Conklin's paper (1917) has been published. In its closing paragraphs he modifies materially the view which he had earlier expressed, admitting that cytoplasmic differentiation of the egg-cell probably arises under nuclear influence exerted equally by the egg and the sperm nuclei of the previous generation, the view maintained by the present writer.

The evidence and a criticism of parts of it follows:

1. Shull's Evidence

(*a*) Cases of maternal inheritance. Under this heading Shull places such experiments as those of Correns (1909) on *Mirabilis jalapa* var. *albomaculata*. This plant —the common four-o'clock—has variegated leaves, green and white, the white being due to inhibited development of green in the chromatophores. The amount of green and white varies in different plants and furthermore whole branches may be green while other whole branches may be white. Flowers borne upon green branches, if self-fertilized, give seed that produces only green offspring. Flowers from white branches, if selfed, give seed that produces only whites, which die because they are unable to carry on photosynthesis. Flowers on variegated branches yield offspring some of which are green, some variegated. Crosses among these green, white and variegated plants reveal the fact that the offspring resemble invariably the female parent. White females pollinated by any green or variegated pollen yield only whites which die. Green females pollinated by white or variegated pollen yield only green descendants. The paternal character never reappears in subsequent generations.

Correns has assumed in explaining these remarkable occurrences that a disease transmitted by the cytoplasm of the ovule is the cause of the color differences, inasmuch as the white color in either self condition or as mottling on the green is a pathological condition. The chromosomes are assumed to be immune to this disease. If the disease is caused by a germ (which is very likely) this germ acts only on the chromatophores and may well be passed through the egg like the germs of syphilis and other diseases. If this is the case, true heredity is not involved. The egg has simply acted as the passive bearer of a foreign body. And if the disease is due to a defect in the chromatophores, the case is not very different. The

chromatophores, as Shull says, are probably "autonomous bodies arising only from other autonomous bodies like themselves." On such a view they are simply structures enclosed within the cytoplasm, having a continuity parallel with but independent of the continuity of inherited characters.

(*b*) Shull's next evidence is drawn from experiments resulting in so-called "matrocline hybrids," which he defines as "unequal reciprocal hybrids which resemble the mother more than the father." Under this head he cites the well-known cases of species and genera crosses among echinoderms. He says of the first generation from sea-urchin ♀ × starfish ♂ (Loeb, 1903), and from sea-urchin ♀ × crinoid ♂ (Godlewski, 1906) that the embryos were purely maternal in character. In contrast to this, other observers of species and genera crosses among echinoderms (Morgan, Boveri, Baltzer and Herbst) have described the F_1 embryos as intermediate between the parents wherever there was normal union of maternal and paternal chromatin. Shull emphasizes especially the maternal character of embryos possessing no maternal chromatin. These were produced by Godlewski, by fertilizing enucleated fragments of sea-urchin eggs with crinoid sperm. The larval stages were reported to have been purely of the sea-urchin type. However, Boveri, Bierens de Haans and Herbst have obtained results which showed either the reverse condition, viz., *paternal* embryonic characters, or else intermediate larvæ. Moreover in the cross fertilization of giant sea-urchin eggs possessing twice the normal amounts of cytoplasm and chromatin, the larvæ inclined to the maternal side. That this was not due to the doubled amount of cytoplasm was demonstrated by Boveri, who fertilized *nucleated* half-fragments of normal eggs with sperm of another species and found no paternal inclination from the halved amount of cytoplasm. That the phenomena are due rather to the chromosomes is indicated by the same experimenter's work with dispermic fertilization. When more than one

sperm enters the egg abnormal mitotic figures and abnormal chromatin distribution are directly correlated with abnormalities in the larvæ, although the egg cytoplasm remains constant. Baltzer's fine work on species crosses may also be cited as showing that Loeb's and Godlewski's observations penetrated only part way toward the truth.

As an example, only one of Baltzer's crosses need be cited. When eggs of *Sphærechinus* were fertilized by sperm of *Strongylocentrotus*, the mitotic figures and distribution of chromatin were normal and the larvæ were intermediate, *not* maternal. From the reciprocal cross "matrocline hybrids" (most of them abnormal) did result, *but* their maternal resemblance was not due to cytoplasm. That it was due to irregularities in the chromatin distribution was proved by Baltzer, who followed the history of the maternal and paternal chromosomes in the hybrid embryos. He found that the majority of the paternal chromosomes (15 out of 18) were inactive at the first cleavage. They were extruded from the developing oösperm nucleus, and degenerated. The cells of the hybrid had then 21 chromosomes, 18 maternal and 3 paternal, and their maternal resemblance is easily explicable on these grounds. It is not explicable on any other for no abnormal conditions obtained in either cytoplasm or the surrounding medium.

Herbst repeated the first of Baltzer's crosses (*Sphærechinus* ♀ × *Strongylocentrotus* ♂) but chemically induced parthenogenetic development in the egg before the entrance of the sperm. Thus the ♂ pronucleus was behind at the first division and failed to be incorporated into the nucleus of one of the first daughter cells. One side of the developing hybrid had, then, merely paternal chromosomes, while the other had both maternal and paternal, and in striking sequence to this distribution were embryos which had only paternal characters on one side, while those of the other side were intermediate.

On the evidence thus far, Shull himself has not placed

the maximum of emphasis, and the foregoing criticism has been intended to indicate that its support of the cytoplasmic view has been and may continue to be so minimized as to be non-existent. However, Shull does place much emphasis on some carefully collected evidence of his own. This does not, I believe, support his theory to any greater extent than his quoted cases.

The evidence is briefly as follows: Shull crossed two lines of rotifers which differed in two egg characters—time of hatching of sexual eggs, and the proportions of sexual egs which actually hatched. The eggs of line "A" hatched on the average in 1–2 weeks, about 50 per cent. emerging. Line "B" eggs took 5–6 weeks to hatch and only 5 per cent. emerged. Line "A" females fertilized by line "B" sperm laid eggs which hatched in 1–3 weeks, 50 per cent. emerging. The eggs thus resembled the mother's line in both respects. Line "B" females fertilized by line "A" sperm laid eggs, 30 per cent. of which emerged in 4–5 weeks, resembling more the mother's line than the father's. The reciprocal hybrids are thus very unequal, says Shull, and since in crossing parents which differ by Mendelian, chromosome-determined characters, the resulting reciprocal hybrids are equal, the characters under observation are non-Mendelian and determined in the cytoplasm of the egg.

But it is to be objected that in reality these hybrid eggs of the first generation are not hybrid in these characters at all. The characters are egg characters and as such can be exhibited only when the hybrid zygote produces its eggs, not when the hybrid zygote is formed. The expression of the character is thus delayed until the hatching of eggs laid by these F_1 zygotes. And Shull's data shows this to be the case.

But when new lines were obtained from these hybrid (F_1) eggs, and these lines produced sexual eggs of their own, the two reciprocal hybrid lines were ***fully equal.***

Now the usual occurrence is observed, viz., the reciprocal hybrids are equal and the contributions to the character

by ♂ and ♀ are proved to be also equal. Shull's contention for the participation of the egg cytoplasm rests entirely on the maternal character of the first egg-generation. These eggs were matured, since the mother was homozygous in the characters, under the influence of the *like* chromatic contributions of her parents; the hybrid mother matured hers under the influence of the *unlike* chromatic contributions of her parents and showed the participation of her paternal chromosomes only in the *behavior* of her eggs. The peculiarities of the case lie not in that we are dealing with a cytoplasmically determined character, as Shull contends, but in (1) the fact that the characters are exhibited only by females; (2) in the fact that the characters are egg-characters, which places segregation one generation farther away from the original cross.

The case is quite analogous to the case of the inheritance of red pericarp color in corn, which, although a "maternal character," was shown to Mendelize by Lock ('06). It is also comparable to the egg-character "unibivoltinism" in silk moths, which Castle ('10) proved from Miss McCracken's data to be a Mendelian character. These cases will be discussed more fully in later paragraphs.

Shull's conclusion that cytoplasm determines egg and larval characters is, I believe, unnecessary. It has been shown that characters exhibited only by females and only in the egg may be equally determined both in the egg and in the sperm. The sperm contribution being predominantly chromatic, and the chromosomes being the accepted carriers of the determiners of other characters, it is to be concluded that the determiners for the characters investigated by Shull are also to be sought in the chromosomes.

Loeb, in his book (1916), has given considerable space to this question, closely following the earlier treatment by Conklin. His conclusion which has been stated and the evidence on which it rests may be briefly criticized by the

addition of further evidence which warrants a changed interpretation of certain facts.

Loeb first calls attention to the exclusively maternal character of the early development of enucleated fragments of eggs when fertilized by sperm of a different species. Such evidence has already been treated above (p. 5).

His second claim—that the *rate* of cell division and development is determined only by the egg cytoplasm—warrants further consideration. An egg from a line in which segmentation of the egg takes place eight hours after fertilization was fertilized by sperm from a line in which segmentation begins in 30 minutes. The rate of these cross-fertilized eggs was 8 hours, like the mother's line.

The careful and long continued work of Newman ('14) has, on the other hand, shown that the entrance of sperm of a different species does materially alter the rate of development.

In both reciprocal crosses between *Fundulus heteroclitus* and *F. majalis* the rate of development of the hybrids was intermediate between that of the two parent species. This was true of cleavage rate, rate of germ-ring formation, etc.

In the cross *F. heteroclitus* × *F. diaphanus* "both reciprocal crosses have a higher rate than the pure bred strain. Similarly, when we make reciprocal crosses between *Cyprinodon* and any species of *Fundulus* we find a marked retardation in developmental rate in both crosses. . . ." It is of the greatest significance that in all three cases the results of reciprocal crosses were equal. Either both were intermediate, both were accelerated or both were retarded regardless of which species was used as the egg-parent. In the face of such evidence, a theory of exclusive control of the egg over early development is untenable.

Newman's fundulus hybrids, while demonstrating the conclusion just stated, do not form critical evidence for determining the action in heredity of such rate-characters because only the F_1 generation is known. Another series

of experiments on a hatching time character has, however, been carried through the F_3 generation and as an illustration such a case may be cited in detail. It consists of Miss McCracken's (1909) experiments with silk moths. Castle later (1910) called attention to some facts in her data which indicated that although a female-exhibited character and confined to the egg in its expression, it nevertheless gave evidence of Mendelizing in crosses. Toyama ('12) concluded that dominance was present, and both of the latter investigators agreed that the males, although unable to exhibit the character, gave evidence by their genetic behavior of having an equal determinative influence with the females. The data follows:

Silk moths lay one batch of eggs, always in the spring. The eggs of some batches hatch out immediately, producing another brood of larvæ and moths in that season. The parents of such batches of eggs are hence known as bivoltins. The eggs of other batches do not hatch for twelve months, and since in this way there is but one brood or flight each season, the parents of such eggs are known as univoltins. If a univoltin female is crossed with a bivoltin male, the spring batch is laid as usual and hatches in 12 months. This is just what would have occurred if the mother had been fertilized by a male of her own sort. When these eggs hatch, a hybrid brood emerges which lay their egg batches immediately but the univoltin character is again exhibited in that all of these eggs are of the 12 months type. But, these eggs now differ among themselves as is shown by the behavior of the zygotes which emerge from them. Some of these females are bivoltin, laying eggs which develop immediately, while others are univoltin, laying eggs which hatch the following spring. The expression of the paternal contribution is delayed, but its activity in determining the time of hatching is quite apparent.

The inheritance of red pericarp color in corn follows exactly the same course as that outlined above, with red dominant over white. The F_2 embryos must be *raised*

before the segregation of pericarp color among them can be seen, for it is exhibited only in the seed coats. The conclusions follow: (1) The egg and all its determinative content is produced under the double influence of the sperm and egg chromatin contributions which united to form the producing zygote. (2) Hybridization experiments with egg characters, to be critical, must be carried as far the F_3 generation. (3) In all experiments which I have seen reported, in which this condition obtained, the influence of the sperm on the characters in question has been observed.

Loeb's evidence, however, introduces also crosses between *Strongylocentrotus purpuratus* and *S. franciscanus*, and the statement is made that the development of the hybrid up to the formation of the skeleton resembles exclusively the development of the mother's species. But Loeb also finds that the cross-barring in the spicules of *purpuratus* behaves as a dominant character in *reciprocal crosses*. He assigns this character to a factor, which he imagines to be a ferment or enzyme. This statement follows: "Since the pure *purpuratus* has two determiners for the development of the cross-bars, and the hybrids only one, the pure *purpuratus* should have twice the enzyme and develop twice as fast"—and it did. He provides here not only evidence that avowed chromosome characters do affect the *rate* of development, but even furnishes an enzymatic mechanism by which they may do it. And yet soon after the above quotation, we read:

> We can therefore be tolerably sure that wherever we deal with a hereditary factor which is determined by the egg alone, the cytoplasm of the latter is partly or exclusively responsible for the result. We have already mentioned that *rate of segmentation* is such a character.

The whole case of the supporters of any theory which views the cytoplasm as determinative rests on either their refusal to go back and inquire the source of this cytoplasm, or on their refusal to give due emphasis to the source, even though they recognize it. Conklin recognizes

the double influence which is exerted on the developing egg better than any of the others who have adopted his "compromise theory." He admits that "most of the differentiations of the cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm." He has demonstrated better than any other one man how complex and definite these differentiations in the egg cytoplasm are. All will agree with him when he says that they "foreshadow" the future organism. But "cytoplasmic organization, while affording the immediate conditions of development, *is itself a result of the nature of the nuclear substance* which represents by its inherent composition the totality of heritable potence." These last are the words of E. B. Wilson (1895, p. 25), although he has translated and adapted them from an earlier paper by Driesch. They represent the opinion of Wilson and of Driesch in full accord. "The nuclear substance" referred to was even then known to contain equality of maternal and paternal chromatin.

Wilson himself had been able to demonstrate that the structure of the cytoplasm in sea-urchin eggs was acquired during ovarian life, and on the basis of this and of a considerable body of similar evidence he was able to conclude quite definitely:

> That a preorganization of the cytoplasm can not be regarded as the primary factor in heredity is conclusively proved by the old argument based on inheritance from the father through the sperm nucleus.

The only link which is needed to make the chain complete is some substantial body of evidence, demonstrating the effect and the mechanism of action of the nucleus on the cytoplasm. This, it must be admitted, has not been entirely filled. Nägeli, to be sure, held a theory of a dynamic effect of the nuclear idioplasm on the cytoplasm, while Driesch contended that the mechanism was chemical. The nucleus, in his opinion, exercised its governance by means of ferments or enzymes. There are facts in

development which point to effects of the nucleus even on the visible differentiation of the egg before fertilization. In the sea-urchin, for instance, this differentiation is preceded by the absorption into the nucleus of part of the fluid content of the cytoplasm, altering the chemical composition of the latter and greatly increasing the bulk of the nucleus. The membrane of this enlarged nucleus then dissolves and part of its contents by their color may be traced to a clear cap of fluid which later gives rise to the skeleton of the echinoderm. Such absorptions and minglings probably play a large part in the reactions of nucleus and cytoplasm, either at the successive disappearances of the nuclear membrane during mitosis or through that membrane.

Nucleus and cytoplasm may certainly be regarded as forming a reaction system analogous to that which might exist between a series of chemical substances (Jennings, 1914). The cytoplasm in turn is linked with the extracellular milieu in a quite comparable way and forms the intermediary between the nucleus and the exterior.

Evidence on this interaction is accumulating. As an example I may quote the work of Cameron and Gladstone on cells other than ovarian, and, to be sure, by the static, histological method. But they have observed fine preparations and have concluded that the cytoplasm is visibly differentiated into two grades of endoplasm. The first is next to the nucleus, is clear and refractive. This is the nascent material of the cell and is the first visible stage in the genesis of protoplasm. It is, they postulate, a derivative of the nucleus itself, and to the nucleus is ascribed the final elaboration of nutritive material which has been ingested by the cytoplasm. This nascent endoplasm they conceive to be the active material of the cell grading into passivity toward the cell periphery, through the maturer endoplasm and the ectoplasm.

Whatever relations may exist between the two, the fact remains that the cytoplasm is necessary. Without it the nucleus, deprived of its milieu, can not live, and develop-

ment can not take place. The investigation of the finer physiological reactions which take place between the nucleus and cytoplasm is badly needed, and the restatement of them in terms of physics and chemistry. Such evidence as is available indicates that the importance of the cytoplasm is, in the main, subordinate to that of the nucleus.

The evidence from egg-characters, it might be noted in conclusion, is one-sided. I have no doubt that if sperm-characters were to be studied as intensively as egg-characters have been (which has not been the case due to the microscopic size of most sperms) the differential characters in the sperm would be found to behave in heredity like the differential characters of eggs, and would be determined as largely by the egg nucleus as by the nucleus of the sperm of the preceding generation.

Conclusions

Direct continuity of substances in the cytoplasm is not a method of heredity. It simply provides for the autonomous proliferation of materials with no determinative significance. No compromise, then, is possible between the two views outlined as "cytoplasmic" and "chromosome" theories of heredity. The first is non determinative; the second is the primarily effective method of heredity and of development. The working of the effective method is known for heredity, if heredity be properly only concerned with the way in which the hereditary factors are distributed in the germ cells. For development, its mechanism is but grossly known, but we have learned enough of the determinative effect of the nucleus and of the possibilities for interaction between cytoplasm and nucleus to foster a suspicion that one day the governance of the chromosomes over development will be explained in physical-chemical terms.

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SHORTER ARTICLES AND DISCUSSION

MODIFYING FACTORS AND MULTIPLE ALLELOMORPHS IN RELATION TO THE RESULTS OF SELECTION

In the prevailing controversy as to the effectiveness of selection, those who reject such effectiveness put forth *multiple modifying factors* as the explanation of the results observed. The given character (for example, the coat color in Castle's hooded rats) is held to depend on one main factor (determining the presence or absence of the character) and upon numerous modifying factors; the number of the latter present in a given case determines the degree of expression of the character. Selective breeding is then held to act, as it does with relation to all other Mendelian factors, merely by making diverse combinations of such factors. Many are gathered together in certain individuals, few in others, and the degree of expression of the inherited character varies accordingly.

Much evidence is presented that this is actually the mode of operation in many cases where selection is effective. Thus are explained the visible results of selection in Castle's rats; thus the unexpected fact that many of the mutations of *Drosophila* have shown themselves (in accordance with Castle's prediction) to be amenable to selection, although in other respects they behave like alterations of a single unit factor. This is indeed the usual explanation for that effectiveness of selection which is coming to light in so many cases; and in many of the cases it appears clear that the explanation is correct.

But in which direction does this explanation carry us? To answer this question we must know what these multiple modifying factors are. If they are mere examples of the static condition of diversity observed among so many closely related organisms, the answer falls in the negative direction, so far as the effectiveness of selection in accumulating actual alterations of hereditary characters is concerned. But if they should themselves turn out to be the actual alterations of hereditary constitution that are accumulated by selection, then the answer con-

firms the effectiveness of selection and adds greatly to our knowledge of how it is brought about. What are the facts?

For these we may turn to the organism of which the genetics are best known; to the fruit fly *Drosophila;* and we may accept the accounts presented by those most uncompromising opponents of the effectiveness of selection, the investigators of *Drosophila* in the Columbia laboratory. Their account we cannot suspect of being colored to favor the selectionist point of view. We find data as to certain known modifying factors in the recent important paper of Bridges (1916) on "Non-Disjunction of the Chromosomes in *Drosophila.*" Bridges tells us that he has found no less than seven diverse factors that modify the single primary grade of eye color known as eosin. These seven factors are located in parts of the chromosomal apparatus different from the spot on which the presence or absence of eosin depends, and each is inherited in Mendelian fashion. One of these factors lightens the eosin color in a fly with eosin eyes, nearly or quite turning eosin to white; this factor Bridges calls "whiting." Another has the effect of lightening the eosin color a little less, giving a sort of cream color; this is called "cream *b.*" A third factor dilutes the eosin color not so much; it is called "cream *a.*" In addition to these, Bridges tells us that he has discovered three other diluters of the eosin color; we will call them the fourth, fifth and sixth diluters. And finally Bridges tells us of another factor whose only effect is to make eosin darker; this factor he calls "dark." We get therefore the following list of the modifying factors for eosin color:

1. Whiting
2. Cream *b*
3. Cream *a*
4. Fourth diluter
5. Fifth diluter
6. Sixth diluter
7. Dark

We have then in *Drosophila* minutely differing conditions of a single shade of color, brought about by seven modifying factors. Concerning these, Bridges makes the following remark, which is worthy of particular attention:

> A remarkably close imitation of such a multiple factor case as that of Castle's hooded rats could be concocted with the chief gene eosin

for reduced color, and these six diluters which by themselves produce no effect, but which carry the color of eosin through every dilution stage from the dark yellowish pink of the eosin female to pure white (Bridges, 1916, p. 149).

Thus we see that in *Drosophila* we could get the same sort of graded results that Castle does with his rats, only in *Drosophila* this is by multiple modifying factors, whereas Castle believes that in the rat it is by actual alterations in the hereditary constitution!

But what are these modifying factors? And here we come to the astonishing point. *These modifying factors are themselves alterations in the hereditary constitution!* Bridges leaves no doubt upon this point. He lists and describes them specifically as mutations; as actual changes in the hereditary constitution.

Now so far as I can see, the question involved in the selection controversy is as to the occurrence of minute changes in the hereditary constitution, and their accumulation by selection; so that by selection various grades of a given external character can be obtained. In *Drosophila*, according to Bridges, such changes occur; changes which give, so far as our present imperfect knowledge goes, at least seven diverse grades of a single tint (that is itself, as we shall note, only one grade in another series of seven known grades). By means of these graded changes one could obtain, by the mutationist's own statement, the continuously graded visible results which selection actually gives. Is not then the controversy as to the effectiveness of selection at an end?

As to just where the graded hereditary changes occur there remain indeed certain differences of opinion; some selectionists, like Castle, hold that the various grades of a given external character are due to diverse minute modifications of the same unit character—of the same locus in the chromosome; while, as we have seen, the modifying factors are due to changes in diverse parts of the hereditary material. This matter of detail does not touch the main point, but it is of interest to ask what the work of the mutationists gives us on this question. It is curious to find that their studies of *Drosophila* furnish almost all that could be asked by the radical selectionist as to the existence of a single unit character in a series of numerous hereditary gradations. The best instance here is again the color of the eye, which furnished us also our example of modifying factors. The color

eosin, of which the modifying factors give us seven grades, is itself only one of another series of seven grades that are due to diverse alterations in the same unit factor—in the same chromosomal locus. As we know from the studies of *Drosophila*, this locus is a certain region of the X-chromosome. When this locus retains its normal condition the eye is red. Some years ago a variation was observed by which the eye lost its red color, becoming white. Somewhat later another variation came, by which the eye color became eosin. By the wonderfully ingenious methods which the advanced state of knowledge of the genetics of *Drosophila* has made possible, it was determined that the mutations white and eosin are due to changes in a particular part of a particular chromosome, and that indeed the two are due to different conditions of a particular region of the X-chromosome. In other words, they show different conditions of the same unit. Moreover the normal red represents a third condition of this same unit.

Later a fourth condition of this same unit was found, giving a color which lies nearer the red, between the red and eosin; this new color was called cherry. We have now four grades of this single unit character.

And now, with the minute attention paid to the grades of eye color, new grades begin to come fast. In the November number of *Genetics*, Hyde (1916) adds two new grades, one called "blood," near the extreme red end of the series; the other, called "tinged," near the extreme white end; in fact, from the descriptions, it requires careful examination to distinguish these two from red and white respectively. So we have now six grades of this unit. And in the same number of the same journal, Safir (1916) adds another intermediate grade, lying between tinged and eosin; this he calls "buff."

So, up to date we know from the mutationists' own studies of *Drosophila* that a single unit factor presents seven gradations of color from white to red, each grade heritable in the usual Mendelian manner. These grades or "multiple allelomorphs" as they are called, are the following:

1. Red
2. Blood
3. Cherry
4. Eosin

5. Buff
6. Tinged
7. White

Three of these seven grades have been made known to us within the last six months. It would not require a bold prophet to predict that as the years pass we shall come to know more of these gradations, till all detectable differences of shade have been made out and each shown to be inherited as a Mendelian unit. Considering that the work on *Drosophila* has been in progress but eight or nine years, we have already remarkable progress toward a demonstration that a single unit character may present as many heritable grades as can be distinguished; that the grades may give a pragmatically continuous series. This is precisely the situation that the selectionist postulates.

Furthermore, as we have seen, besides this primary series of seven grades, due to alterations of a single unit factor, there is a secondary series containing seven more grades, all affecting the central grade (eosin) of this primary series, but due to alterations of other parts of the germinal material. How much more does the selectionist require?

This situation in *Drosophila* is not exceptional. To mention one or two other examples, Castle and Wright (1916) find a large series of such diverse conditions of a single factor (''multiple allelomorphs'') determining various shades of coat colors in rodents. Emerson (1917) in his recent account of the extraordinary condition of affairs in the genetics of pericarp colors in corn, talks of ''a series of not less than nine or ten multiple allelomorphs,'' which moreover leap back and forth from one condition to another in bewildering fashion.

To sum up, it appears to me that the work in Mendelism, and particularly the work on *Drosophila*, is supplying a complete foundation for evolution through the accumulation by selection of minute gradations. We have got far away from the old notion that hereditary changes consist only in the dropping out of complete units, or that they are bound to occur in large steps. The ''multiple allelomorphs'' show that a single unit factor may exist in a great number of grades; the ''multiple modifying factors'' show that a visible character may be modified in the finest gradations by alterations in diverse parts of the germinal material. The objections raised by the mutationists to gradual change through selection are breaking down as a result of the thoroughness of the mutationists' own studies.

The positive contribution of these matters to the selection problem is to enable us to see the important rôle played by Mendelism in the effectiveness of selection. Hereditary variations, such as give rise to the multiple allelomorphs and multiple modifying factors, occur in some organisms rather infrequently, as measured by the time scale of human happenings. If there were no interchange of factors among individuals and stocks, it would take a long time to obtain in one individual all the six diluters of the eosin color of the *Drosophila* eye; one arises in one individual, another in another. But by selective crossbreeding it is possible to bring together into one stock all the modifiers that have been produced in diverse stocks. Mendelism acts as a tremendous accelerator to the effectiveness of selection.

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A WING MUTATION IN PIOPHILA CASEI

IN the early part of December, 1915, I began to breed the "cheese skipper" *Piophila casei,* in order to see if mutations were to be found in this fly. The source of my stock was a small piece of Italian cheese containing a dozen or so larvæ.[1] As these were doubtless the offspring of one female, inbreeding has been very close. Up to June 22, 1916, only one heritable mutation had been found among the thousands of individuals bred; this was the wing defect described below, which was first noted on March 12, 1916.

[1] This work was carried on at the Osborn Zoological Laboratory, Yale University, New Haven, Connecticut. It was in New Haven that I obtained the cheese. Contribution No. 135, Zoological Laboratory, University of Texas.

The Defect.—When viewed from the dorsal surface the defect appeared as a blister of variable size on the proximal and posterior part of the wing. From the ventral surface it appeared as a pit. Occasionally a real blister filled with fluid was obtained. The position of the defect was constant; when small it lay in the posterior cell just below the discal cell. When large it involved nearly the whole wing including the axillary, anal, second basal, discal and posterior cells. Usually both wings were affected alike, but here and there flies were found with one wing normal and the other wing severely affected.

This factor is strikingly similar, both in its appearance and the variability of its behavior, to the "balloon wings" found by Morgan[2] in *Drosophila* and more recently fully described by Marshall and Muller.[3] The flies carrying the defect, in my cultures, were very frequently sterile, and in no case did their fertility begin to approach that of normal stock.

In breeding, the character behaved as a mendelian recessive. Normal crossed with balloon gave, in the F_1 generation, 196 normal and no affected individuals. (This included 4 matings.) When brother and sister were mated, in the F_2 generation, 312 normal and 111 balloon offspring were obtained This is very close to the expected 3:1 ratio, of a monohybrid cross. When balloon flies were crossed, all individuals were affected (74 offspring obtained) but the character showed itself extremely variable; in some cases the flies appeared normal until very closely examined.

The defect was not sex-linked as is shown by the following mating. A defective female was mated with a normal male of normal stock. Of the 50 offspring resulting both males and females were normal.

The variation in the appearance of the balloon flies suggested either that the size of the blister was dependent upon some unknown environmental factors, or else, was due to multiple allelomorphs or multiple factors. A great number of matings were made to gain light on this point, but due to the sterility of the affected individuals, the evidence is not sufficient to allow us to draw any conclusions. Two individuals both of whom were severely affected were crossed. The 20 offspring resulting were all severely affected. Two individuals, both of whom were only

[2] In Morgan's "A Critique of the Theory of Evolution."
[3] Marshall and Muller, *Jour. of Exp. Zool.*, Vol. 22, 1917.

slightly affected, were crossed. Of the 29 offspring resulting, 17 showed the defect in a severe form, and 12 showed only small blisters. A female which had only one wing affected, was mated to a male, one of whose wings was severely affected while the other bore a very small blister. Of the 45 offspring resulting, 27 bore the defect in a severe form on both wings and 18 showed small blisters again on both wings.

Further experiments with this new character were under way when the work was stopped by the mobilization of the Militia in June. The work with these flies, however, is again being resumed.

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A CASE OF REGENERATION IN PANULIRUS ARGUS[1]

THE occurrence of regenerative processes in the crustacea has been a matter of record for a number of years, but the instances have been mostly confined to the regeneration of appendages and portions of the nervous system. Observations on the regeneration of portions of the exoskeleton of the trunk are far less numerous. The present observations on the regeneration of a portion of the rostrum of *Panulirus argus*, the common crayfish of the Bermuda Islands, were made during the summer of 1916 at the Bermuda Biological Station.

Panulirus argus when full grown is about 14 to 16 inches in length. It lacks chelipeds, their place being taken by the ordinary type of walking appendage. None of the walking appendages is provided with nippers, all being tipped with a single hook, as, *e. g.*, in the fourth pair of appendages of the crayfish *Cambarus*. The rostrum of *Panulirus*, instead of being a single median projection, consists of a pair of long (30–35 mm.), sharply pointed spines, slightly compressed laterally, and growing out from the carapace just posterior and slightly dorsal to the base of the eye-stalks.

The animal in question was a half-grown male, eight and one half inches long. When caught, June 20, the left spine (compare figure and explanation) of the rostrum was entirely missing. The carapace around the base was jagged and rough, as though the break had been recent; but a thin, soft membrane had

[1] Contributions from the Bermuda Biological Station for Research, No. 58.

formed across the surface of the break. Five days later, June 25, the protecting membrane had hardened, so that it could not be dented with the point of a scalpel. No further change could be noted until after the molting, which occurred four days later, June 29. The casting occurred at night, and the next morning the new shell showed no signs of any wound. By one o'clock a very slight hump appeared, and by ten o'clock at night a little rudimentary spine 2 mm. in length had formed. The next morning another millimeter had been added to its length. Meantime the normal spine had increased 1.5 mm. in length. No further growth followed before the new shell had hardened.

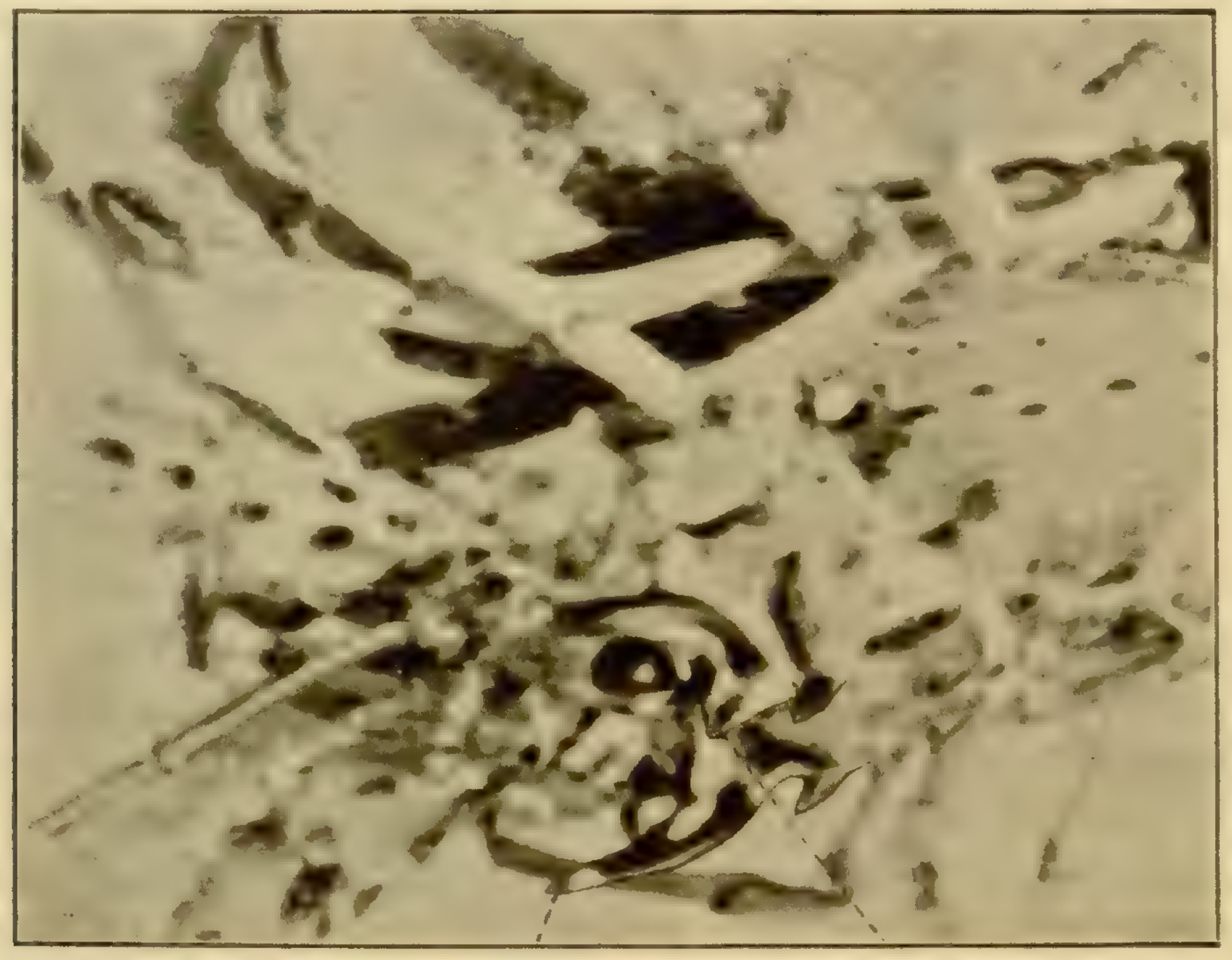

FIG. 1. From a photograph of the left side of the head region of *Panulirus argus*, showing (*norm.*) the normal, and (*regn.*) the regenerated rostral spine. As the figure is reproduced from a photographic print, the picture is reversed, the right spine appearing like a left one.

Sixteen days later, July 15, another molt occurred. As before, the old shell was cast at night and by the following morning the regenerating spine had added 2 mm. to its length, being now 5 mm. long. By the next evening all growth had been stopped by the hardening of the new shell, but the total length of the spine was at this time 7 mm. The spine now showed a sharp point and also a slight lateral compression like that of the normal spine. At this casting the normal right spine added 1 mm. to its length, showing that, while the whole animal was growing, the

regenerating part was increasing at a much faster rate than other parts.

Thus in the period of twenty-seven days during which the animal was under observation, it had undergone two molts and had regenerated a missing rostral spine of normal form, 7 mm. in length, while the normal spine had added 2.5 mm. to its length in the same period. These results show that the period between molts for this animal under laboratory conditions is sixteen days; that a rostral spine of normal form can be regenerated; and that the rate of this regeneration was nearly three times the rate of normal growth of a similar spine during the same period.

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NOTES AND LITERATURE

THE COAL MEASURES AMPHIBIA OF NORTH AMERICA[1]

The excellent monograph by Dr. Roy L. Moodie is a worthy successor of the long series of works by Dawson and by Cope on the air-breathing vertebrates of the Coal Period in North America. The extremely varied amphibian fauna of the coal swamps as described by Moodie contains representatives of no less than 7 orders, 19 families, 46 genera and 88 species, the animals ranging in size from the minute *Eumicrerpeton*, less than two inches long, to the great *Leptophractus obsoletus*, which was as large as an adult Florida alligator. The wide differentiation and high specialization of these amphibians shows that the class even at that early epoch had evolved very far from its first adaptive radiation, so that, as Dr. Moodie well observes, the origin of land vertebrates from fishes must be looked for in a much earlier time, perhaps the Silurian.

Carboniferous Amphibia are reported from various localities in North America, but only four of these have yielded large or important collections. From the South Joggins coal mines in Nova Scotia Sir William Dawson secured most of the specimens of Microsaurs described by him, many of the skeletons being found in the rotten stumps of *Sigillaria* trees. This material is preserved chiefly in the Museum at McGill University, Montreal. From the Linton, Ohio, coal seams Newberry and his collectors secured the great collections described by Cope and which are now chiefly in the American Museum of Natural History. At Mazon Creek, Illinois, the fossils are found in ironstone nodules in a stratum of shale; the specimens have been described chiefly by Newberry, Cope and Moodie and are scattered in various museums. At Cannelton, Pennsylvania, the fossils occur in slates and have been described by Moodie, the material being in the National Museum.

On account of the fragmentary nature of most of the material and the fact that generic and specific names have been based on

[1] Carnegie Institution of Washington, Publication No. 238, 1916.

many different and non-comparable parts of these animals, the author's task was an exceedingly difficult one, and only those who have occasion to study this work very closely can appreciate either the magnitude of the undertaking or the thoroughness with which it has been carried out.

Dr. Moodie's monograph will naturally invite comparison with the well-known works on the Permian Amphibia of Bohemia and Saxony by Fritsch and by Credner. It must be admitted, however, that many of the illustrations are inferior to those of the works mentioned, partly on account of the difficulty of showing the real character of these fragmentary specimens by means of photographs.

The author's method is so intensive that he has left even readers who may have some first-hand knowledge of Paleozoic Amphibia in need of many broader facts and comparisons which may reasonably be expected to result from such a conscientiously executed investigation; it is the aim of this review in some measure to supply this deficiency, in the hope that Dr. Moodie himself may be induced to write a general article covering more fully the points here raised.

In the chapter on stratigraphic and geographic distribution the author shows that the four chief Amphibia-bearing formations in North America mentioned above are all in the Allegheny or Lower Coal Measures and are thus much older than those deposits (Salt Fork, Pitcairn) of the Upper Productive Coal Measures at the top of the Pennsylvanian series, which have collectively yielded *Cricotus, Diplocaulus, Eryops* and other genera characteristic of the Texas "Permian."

The author does not discuss the faunistic relations of the Lower Coal Measures fauna either with the "Permian" fauna of Texas and other states, or with the Carboniferous and Permian faunas of Ireland, Scotland, England, France, Saxony and Bohemia. Even the Permian and Triassic amphibian faunas of South Africa invite comparison with the varied Temnospondyli of the Carboniferous and Permian of America and Europe.

The Lower Coal Measures fauna of America includes a long series of branchiosaurs, microsaurs and primitive labyrinthodonts (*Spondylerpeton, Dendrepeton, Macrerpeton, Eobaphetes*), and it is totally lacking in pelycosaurs, poliosaurs, cotylosaurs, or any other reptiles except *Eosauravus*. The Texas fauna, on the other hand, has only a single microsaur (*Crossotelos*) and no branchiosaurs; its varied labyrinthodonts

(including *Cricotus, Eryops, Dissorophus* and many others) are all genera not found in the Lower Coal Measures, and it abounds in reptiles of several orders and many families. Some of this difference may be due to the fact that the Lower Coal Measures fauna represents only the life of the coal swamps, while the Texas fauna represents the life of the pools and streams of a wide delta country (Case); but all authorities agree that the former is much the older of the two.

The Lower Coal Measures fauna is far more similar to the Permian fauna of Bohemia, which according to Fritsch's classification comprises a similar series of 13 families, 26 genera and 63 species, of branchiosaurs, microsaurs and temnospondyls. But no genera are common to the two countries and many of the "families" (as listed) are peculiar to one or the other. The families peculiar to America are the Cocyctinidæ, Peleontidæ, Tutidanidæ, Ptyoniidæ, Molgophiidæ, Sauropleuridæ, Amphibamidæ, Ichthycanthidæ, Stegopidæ, Macrepetidæ, while those peculiar to Europe are the Apateonidæ, Limnerpetidæ, Microbrachidæ, *Dolichosoma, Ophiderpeton,* Melosauridæ and Archegosauridæ. The families common to both continents are: Branchiosauridæ, Diplocaulidæ,[2] Hylonomidæ, Urocordylidæ, Nyraniidæ,[3] Cricotidæ (Diplovertebridæ), Anthracosauridæ, Mastodonsauridæ.

Professor Case has directed attention[4] to the marked resemblance of two of the genera (*Diplovertebron, Macromerion*) from the lowest Bohemian horizon (Nyran) to *Cricotus* of the Upper Coal Measures of North America, as furnishing evidence that the Bohemian deposits are of Upper Carboniferous age.

Subsequent research may well show on the one hand that some of the American "families" are more closely related to European groups than is now recognized and on the other hand that some of the "families" classed as common to both continents are artificial or ill defined (Hylonomidæ?, Nyraniidæ?); yet even with our present imperfect knowledge it appears that the Lower Coal Measures fauna of America and the "Permian" fauna of Europe represent nearly identical life conditions and similar

[2] *Brachyderpeton* of the English Coal Measures, as shown by Watson, appears to be related to *Diplocaulus.*

[3] The presence of this family in America is doubtful, and Dr. Moodie's reasons for assigning the genera *Ichthyerpeton* and *Cercariomorphus* to this family are not stated and difficult to infer.

[4] *Science,* Vol. 42 (Dec. 3, 1915), pp. 797–798.

adaptations on the part of two divergent associations derived from some older and common source, possibly of Mississippian age and of wide distribution; and it further appears probable that the American Lower Coal Measures fauna is somewhat the older of the two.

The chapter on the morphology of the Coal Measures Amphibia contains a careful description of the characters of the skull and other parts of the skeleton, but the author is extremely chary of generalizations. He might have mentioned, for instance, the interesting fact that the skull-pattern of these amphibians is a shifting mosaic, one in which several of the dermal elements have different contacts and different positions in the various families. In some microsaurs, for example, the postorbital grows backward and secures a broad contact with the tabular; in others it retains its primitive position. The jugal and lacrymal also differ widely in their form and contacts. The nasals and adjacent elements are small and much crowded in many branchiosaurs and microsaurs, long and wide in most labyrinthodonts. Certain dermal elements are present in some and absent in others, especially the intertemporal and the rare interfrontal and internasal elements. The shape of the occiput differs widely, sometimes truncate posteriorly, with the auditory notch obsolete, sometimes angulate posteriorly, retaining the primitively wide auditory notch. Very curious is the tendency of the different families of microsaurs to develop "horns"—sharp backwardly projected apophyses in the occipital region—growing sometimes from the tabular, sometimes from the squamosal and sometimes from both at once. These remind one of the backwardly directed processes from the "epiotic" and supraoccipital in the skull of teleost fishes and perhaps they may have served for the attachment of longitudinal ligaments or muscles in wriggling, aquatic types.

All the differences in skull pattern may be regarded as minor readjustments which were taking place after the more profound transformation of a generalized pro-ganoid skull into the amphibian type, the greatest alteration including the loss of the opercular bones so as to leave the gill chamber covered only by membrane and the change of the preopercular or cheek plates into the squamosals. The author expresses the opinion that the membrane bones may have originally been derived from scales "which later became consolidated into large bony scutes," but on histological grounds the reviewer regards it as far more prob-

able that in the ancestral fishes each membrane bone and each scale grew from clusters of cosmine tubercles underlain by tracts of vascular and stratified bony tissue, and that there never was a time when the elements of the dermo-cranium were scale-like in form (*i. e.*, rhombic or polygonal), although the several tissues involved were histologically identical in the body scales and in the dermo-cranium.

On page 85 the author uses the name "squamosal" for the element which he and most other authorities now designate as "supratemporal."

In the description of the hyobranchial elements of *Coccytinus* (a genus doubtfully assigned to the Proteida), the reader looks in vain for a comparison with the same elements in the Permian "Urodele" *Lysorophus* as described by Williston. It may be noted, by the way, that the branchial arches in that genus are extremely primitive and almost *Polypterus*-like in form and arrangement, although doubtless homologous also with those of the modern *Amblystoma.*

The author has given a very thorough study of the dermal scales and scutes of the branchiosaurs, microsaurs and temnospondyls. The ventral "scutellæ," which appear to be homologous with the abdominal ribs of reptiles, are formed, the author holds, as ossifications in the connective-tissue septa or myocomata of the ventral muscles, vestiges of these having been found in modern urodeles. The highly differentiated characteristics of this ventral armature affords many family and generic characters; it is sometimes absent or reduced to needle-like ossicles, sometimes highly developed, forming heavy median V's and wide lateral shelves (*Ctenerpeton*). Some of the microsaurs had rounded, slightly imbricating fish-like body scales with concentric markings which recall the similar armature of certain Bohemian and Saxon types, such as *Ricnodon* and *Discosaurus.* Vestiges of such conditions may be represented in the scales of modern cæcilians (as shown in the enlarged figures of cæcilian scales by the Sarasin brothers).

The reviewer ventures to doubt the correctness of Dr. Moodie's reconstruction of the shoulder-girdle of branchiosaurs and microsaurs, in the matter of the position of the scapula. Many of the specimens figured by Fritsch and by Credner seem to indicate that the concave border of the scapula was posterior in position, as it is in *Eryops* and in modern urodeles, and that it did not form the glenoid border as in Dr. Moodie's reconstructions.

Dr. Moodie's history of the classification of the Amphibia appears to the reviewer to be rather meager, since he simply lists the classifications of his predecessors without giving any critical discussion. It is surprising that in this chapter he did not mention the work of Fritsch with which he must be extremely familiar. Fritsch's classification of the extinct Amphibia, although it was adapted and extended from the classification proposed by the British Association Committee in 1870, was, in the judgment of the reviewer, a distinct contribution to the subject which certainly deserves notice in an historical review, especially since Fritsch erected several new families and gave definitions of all the European groups.

The author's own classification is an interesting attempt to divide the Amphibia of the Coal Measures into two major series or subclasses, the first (Euamphibia) including all those which may be related to modern types; the second comprising all the wholly extinct groups (microsaurs, aistopods and labyrinthodonts of all suborders). He derives most of the modern urodeles (Caudata) from the branchiosaurs, for which he has given considerable evidence; he follows Cope in provisionally deriving the modern Proteida from the Coccytinidæ of the Coal Measures. He regards the strange *Diplocaulus*, an amphibian with a head like a colonial cocked hat, as a member of the Euamphibia, probably because its vertebræ bear short, straight, double-headed ribs which are attached to paired lateral apophyses springing from the middle of the vertebræ, after the fashion of those of branchiosaurs and Caudata and quite unlike the hour-glass centra of microsaurs, which bear long, slender ribs between the vertebræ. But Watson and Williston regard *Diplocaulus* and *Brachyderpeton* as microsaurs, the last named genus showing in the vertebræ and in the skull how the *Diplocaulus* type may have been derived from primitive microsaurian conditions. Indeed it may well be argued that the branchiosaurs and urodeles (Caudata) themselves, in spite of the retention of gills in the young, may have been derived from primitive microsaurs, that is that the vertebræ and ribs of microsaurs are on the whole much more primitive than those of branchiosaurs and Caudata.

The systematic relations and origin of the frogs and toads remain doubtful. Dr. Moodie gives an excellent discussion of the resemblances of *Pelion lyelli* Wyman, from the Linton, Ohio, Coal Measures, to the modern Anura but leaves the phylogenetic problem open. *Pelion* is so little known that it may or not be

ancestral to the Anura, and the Jurassic Anura are so entirely modernized that they do not bridge over the wide structural gap between the Paleozoic Amphibia and the modern frogs and toads. It seems to the reviewer, after repeated comparisons of the osteology of the Anura with that of many of the temnospondyls, that some members of the latter group, in the brain-case, the dermo-cranium and even in the vertebræ and limbs retain many characters which may reasonably be looked for in Paleozoic ancestors of the frogs and toads; and that such forms as *Brachyops*, *Cacops* and *Dissorophus*, although not directly ancestral, differ from the Anura chiefly in the retention of many primitive amphibian characters. It may be that some of the short-headed Triassic temnospondyls of South Africa will furnish the linking forms; but at any rate it is interesting to note that the existing frogs and toads retain a long series of characters in the skull and skeleton which are seen in the Paleozoic temnospondyls, and that they differ from the latter in such modernized characters as the following: the wide fenestration of the occiput and palate, the resulting slenderness of the skull bones, the loss of the dermosupraoccipitals, tabulars, ectopterygoids, pre- and post-frontals, the completion of the auditory ring, the development of extreme saltatorial adaptations in the skeleton, including the modification of the vertebræ from the rhachitomous into the notocentrous and epichordal types, the development of a long continuous urostyle coincident with the forward shifting of the sacrum and lengthening of the ilium.

Dr. Moodie's arrangement and sequence of the families of microsaurs appear to the reviewer to be highly confusing. It would perhaps have been better, after beginning with the newt-like types, to pass at once to the long-bodied Urocordylidæ and the snake-like Molgophiidæ and Ptyoniidæ, instead of interjecting in the middle of the series the Stegopidæ, which appear to the reviewer to be more nearly allied with the Temnospondyli, and the Amphibamidæ, which are heavy-limbed offshoots of the primitive microsaurs.

The author's ordinal and family definitions are extremely full, but the reader will find so many characters that are common to several families and sometimes orders, that it is difficult to cull out the most striking ones. This the reviewer has attempted to do in the subjoined table in which he has also included the principal European families of branchiosaurs and microsaurs. The families of microsaurs are arranged so far as pos-

sible in the general order of their specialization, proceeding from the more primitive newt-like forms to the snake-like microsaurs or Aistopoda.

SYNOPSIS OF THE PRINCIPAL BRANCHIOSAURS AND MICROSAURS OF AMERICA AND EUROPE

A. Vertebræ phyllospondylous, *i. e.*, having the notochord expanded in the middle of each vertebra; transverse process in dorsal region large; ribs short, straight and heavy and borne on the transverse processes, usually near the middle of the vertebræ. Skull broad, obtusely rounded.

B. Auditory notch posterior rather than lateral in position.

Branchiosauridæ.

BB. Auditory notch extended laterally, the squamosals lying far forward .....*Apateonidæ.*[5]

AA. Vertebræ lepospondylous, *i. e.*, with the centra forming gently constricted cylinders; ribs intercentral, typically long and curved.

B. Digits (when present) 4 in manus, 5 in pes; carpus and tarsus cartilaginous. ..

C. Body newt-like.

D. Body covered with cycloid scales or sculptured scutes. Hind limbs longer than fore limbs.

E. Skull narrow .....*Hylonomidæ.*

EE. Skull broad.

F. Ribs short, slightly curved.

Limnerpetidæ.[5]

FF. Ribs thin, curved .....*Microbrachidæ.*[5]

DD. Body-scales reduced or absent; neural and hæmal spines of caudal vertebræ often expanded.

E. Ventral scutellæ absent, tail moderate in length, skull without "horns."

Tutidanidæ.

EE. Ventral scutellæ weak or moderately developed, tail long.

F. "Horns" on squamosals.

Diceratosaurus.

FF. "Horns" on tabulars. *Urocordylidæ.*

Brachiderpeton.[6]

Keraterpeton.[6]

EEE. Ventral armature highly developed, consisting of rods, plates or stout bristles. Skull (so far as known) without "horns." Ribs broad and heavy. Limbs well developed with claw-like phalanges .....*Sauropleuridæ.*

F. Skull very wide and obtuse; teeth heterodont .....*Saurerpeton.*

[5] Permian of Europe.

[6] Europe.

FF. Skull moderately elongate; teeth homodont. *Sauropleura.*

FFF. Skull very large; teeth with acute compressed apex and anterior cutting edge. *Leptophractus.*

FFFF. Skull unknown; abdominal ribs very heavy with broad shelf-like lateral extensions; spines of vertebræ pectinate as in *Urocordylus*, *Œstocephalus* and *Ptyonius*. *Ctenerpeton.*

CC. Body *Proteus*-like (serpentine). Limbs much reduced or absent .....(*Aistopoda*).

D. Ventral armature weak or absent.

E. Ribs long, heavy and broad; neural and hæmal spines short or absent ....*Molgophiidæ.*

EE. Ribs delicate, single-headed; neural arches wide with low spines. Skull narrow, pointed. *Dolichosoma.*[7]

EEE. Ribs well developed; neural and hæmal spines of caudals expanded, pectinated; skull lanceolate with long, slender teeth ...*Ptyoniidæ.*

DD. Ventral armature consisting of narrow, oat-shaped scutellæ; ribs forked, two-headed; neural arches with low spines, lower transverse process expanded into a wide plate in the anterior half of the vertebra. Skull shorter and blunter than in *Dolichosoma*. *Ophiderpeton.*[7]

CCC. Body stout; limbs well developed (but still with cartillaginous carpus and tarsus). Tail short, head very large. Ribs long.

D. No "horns" on squamosal, skin covered with rounded or hexagonal tuberculated scales. Ventral scutellæ present .....*Amphibamidæ.*

DD. Squamosals produced into "horns." Ventral scutellæ apparently similar to those of *Amphibamus*. *Eoserpeton tenuicorne.*[8]

BB. Digits of manus unknown; pes with well ossified tarsus. (Limb suggests *Erypos*). Ventral scutellæ delicate. Centra amphicœlous, spines broad and heavy .....*Ichthycanthidæ.*

AAA. Vertebral centra discoidal.

B. Vertebræ short, thick and probably amphicœlous. Body covered with small cycloid scales .....*"Nyraniidæ"* (?).[9]

C. Body newt-like .....*Ichthyerpeton bradleyi.*
Cercariomorphus.

[7] Europe.

[8] Placed by Dr. Moodie in the Urocordylidæ, but possibly related to *Amphibamus*.

[9] The skull of *Nyrania* as figured by Fritsch (Vol. II, p. 34) appears to the reviewer to relate this genius with the Temnospondyli; but it is placed provisionally by Dr. Moodie in the Microsauria.

CC. Body *Proteus*-like .....*Ichthyerpeton squamosum.*

BB. Vertebræ checker-like, deeply amphicœlous. .*Eosaurus.*

AAAA. Vertebræ unknown. Skull of pretty generalized type with separate intertemporal. Lacrymal and nasals large, orbits central rather than anterior; squamosals produced into short, divaricate "horns." .....*Stegopidæ.* (*Stegops*).[10]

[10] May be remotely related to the Temnospondyli (W. K. G.).

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BIOLOGICAL ENIGMAS AND THE THEORY OF ENZYME ACTION

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I

DURING the past twenty years the sciences which deal with inorganic physical phenomena have made astounding progress in the logical synthesis of their facts and theories.[1] The beginnings of this synthetic tendency lie, of course, in the middle part of the last century, in the work of such men as Faraday, Maxwell, and Mendelejeff. The discovery of radio-activity by Becquerel in 1896, and the demonstration by Thomson of the electron, in the following year, let loose the pent-up forces of an intellectual avalanche which swept scientific conservatism quite off its feet, and seems to be carrying our thought with thrilling rapidity towards a goal which metaphysical philosophers have for ages regarded with wistful longing. This goal is the comprehension of the physical universe in terms of a few simple conceptions.

The lines of demarcation which once were so rigidly drawn between the departments of physical science are disappearing before our eyes. The discovery of radio-activity, instead of adding a new science to the list, has

[1] D. F. Comstock and the present writer have attempted to give an elementary, but comprehensive presentation of the modern theory of matter in their book, "The Nature of Matter and Electricity," 1917.

brought us very close to a cancellation of all of the names except one; the demonstration of the existence of particles of negative electricity smaller than any known atom, instead of further complicating the facts of chemistry, has introduced a hundred simplifications. Mechanics, chemistry, optics, and the sciences of heat, electricity and magnetism are rapidly fusing into a single logical system, the ultimate terms of which are minute particles of positive and negative electricity, the ultimate laws those of electro-dynamics, and the ultimate problems those of the structures formed by these particles in space and of the changes which these structures undergo in time.

This startling progress in physics during the last two decades has not been the product of unadulterate empirical research. On the contrary, it has been made possible only by acts of daring speculation, which to certain more orthodox scientists of an earlier period might have seemed inexcusably foolhardy. However, their justification has often come so quickly and in such unequivocal terms, that methodological critics have been obliged to remain modestly silent. To indulge in a definite and detailed account of the structure and behavior of single atoms of hydrogen—particles far beyond the visual range of even the ultra-microscope—may seem no more a scientific undertaking than the fabrication of a fairy-tale; and yet when from this account there emerges by inevitable logic a mathematical formula corresponding exactly with the constitution of the complex spectrum of hydrogen, our minds are opened to the possibility that the speculation is pointing the way to a fundamental truth.[2] This impression becomes especially forcible when we consider that the constitution of this same hydrogen spectrum had for twenty-eight years defied the intellects of the best scientists, and by some had been regarded as incapable of explanation upon any simple hypothesis.

It is a fact of fundamental logical significance that the

[2] The reference is to the theory of N. Bohr, published in the *Philosophical Magazine*, 1913, 26; 1, 476 and 857.

progress represented by the modern electro-molecular conception of the physical universe has been achieved by the utilization of *a few general conceptions*, such as those of the electron and electrical action at a distance, These conceptions, although general, *i. e.*, universally applicable, are nevertheless extremely definite. They are also as tangible, or concrete, as it is possible to make them. It is nearly as characteristic of the modern theory of matter to eliminate abstractions as it is for it to gather up scattered facts and theories to unite them into an integral system. Although elements of abstraction still remain, they are reduced to a minimum by the increasing tendency to demand not only an algebraic symbol, but a visual picture of the processes of nature.

II

It is perhaps not surprising that the astonishing progress of general physics during recent times should thus far have failed to exert any very notable influence upon the science of biology. From the point of view of the physicist, biological problems must be regarded as questions of special material structure, usually of a very intricate character, and involving the arrangement and history of units of matter for the most part larger than those upon which his attention is immediately concentrated. The program of modern physics is to build up the theory of all material structures by means of geometry and the dynamics of electrical particles. The first problem, logically, is that of the constitution of the atom, and as the solution of this problem is still unfinished, too much should not be expected of our knowledge of the configuration of particles and forces in higher aggregates of matter.

However, a critic who sees current events in the light of the history of science can hardly escape a twinge of disappointment at the recrudescence in biological theory, at the present time, of the doctrine of *vitalism*. The present, of all periods in the history of thought, is an hour of triumph of the monistic theory of nature, and yet now,

more frequently than during the nineteenth century, men eminent in biology seem to quail before the complexity and delicacy of the life process, and, while uttering mechanistic truths about life, to offer them as sacrifices to a spirit of vagueness and discouragement.[3]

It is my belief that this rejuvenation of mysticism and Aristotelian teleology is due not so much to a natural admiration on the part of biologists for obscure ways of thinking, as to their neglect of modern physics and of the methods of thought pursued in that science. It is the purpose of this paper, which is intentionally polemical in manner, to rebuke this tendency by commending to the attention of biologists a general speculation concerning the life process, which—although incapable of immediate verification in all of its aspects—does answer the most perplexing questions raised by vitalism, and at the same time forms a perfectly distinct bond between biological theory and the modern theory of matter.

It is not improbable that the future will look back upon contemporary theoretical biology as a reactionary phase in the history of the science. The great synthetic energy of the Darwinian theory has been spent, has accomplished its magnificent results, but has left many tattered ends, by means of which a few of its enemies are attempting to tear down the entire structure once more. Even the remarkable discoveries which are classed under the name Mendelism are sometimes turned against the mechanistic conception of evolution. These discoveries, although patently of fundamental importance for the theory of life-processes, have as yet provided us with no new synthetic instruments of thought, but instead have generated an amazing and ever-growing list of abstract concepts. However, these concepts do furnish us with a means for the analysis of species in terms of their genetic determination and the recent studies of Morgan[4] and Goldschmidt[5] in

[3] Consider, for example, the contents of Haldane's recent address on "The New Physiology," *Science* (1916), N. S., 44, 620–632.

[4] Morgan, T. H., and others, "The Mechanism of Mendelian Heredity," 1915.

[5] See Goldschmidt, R., "Genetic Factors and Enzyme Reaction," *Science*

this field are pointing the way to synthetic considerations of far-reaching significance.

That biologists recognize the need of new light in the theory of heredity and of evolution, is clearly shown by the following quotations, from Bateson's Silliman lectures:

> In spite . . . of the general attention devoted to the study of variation and the accumulation of material bearing on the problem, no satisfactory or searching classification of the phenomena is possible. The reason for this failure is that a real classification must presuppose knowledge of the chemistry and physics of living things which at present is quite beyond our reach. It is however becoming probable that if more knowledge of the chemical and physical structure of organisms is to be attained, the clue will be found through genetics, and thus that even in the uncoördinated accumulation of facts of variation we are providing the means of analysis applicable not only to them, but to the problems of normality also.

Again:

> Somewhat as the philosophers of the seventeenth and eighteenth centuries were awaiting both a chemical and a mechanical discovery which should serve as a key to the problems of unorganized matter, so have biologists been awaiting two several clues. In Mendelian analysis we have now, it is true, something comparable with the clue of chemistry, but there is still little prospect of penetrating the obscurity which envelopes the mechanical aspect of our phenomena.

Again:

> When with the thoughts suggested in the last chapter we contemplate the problem of evolution at large, the hope at the present time of constructing even a mental picture of that process grows weak almost to the point of vanishing. We are left wondering that so lately men in general, whether scientific or lay, were so easily satisfied. Our satisfaction, as we now see, was chiefly founded on ignorance.[6]

It will be perceived that the demand made by Bateson in these passages is not for new biological facts, but for physico-chemical conceptions in terms of which a chaos of biological facts, already at hand, can be explained, or systematized. Moreover, the emphasis is laid entirely upon the inability of the mind to conceive an explanation,

(1916), 43, 98–100. Also "Experimental Intersexuality and the Sex Problem," AMERICAN NATURALIST (1916), 50, 705–719.

[6] Bateson, W., "Problems of Genetics" (1913), 31, 32, and 97.

or a synthesis of these facts, rather than upon the necessity of detailed proof of some explanation which has already been offered. The contents of genetics would verify the proper conceptions if the human mind were only capable of suggesting them.

In another place,[7] Bateson says, with reference to the mechanism of cell division:

> It is, I fear, a problem rather for the physicist than for the biologist. The sentiment may not be a popular one to utter before an assembly of biologists, but looking at the truth impersonally, I suspect that when at length minds of first rate analytical power are attracted to biological problems, some advance will be made of the kind which we are awaiting.

As a matter of fact, in the school of the physical chemists there has been in preparation, since the days of Thomas Graham, a system of knowledge which, even in its present unfinished form, has a most important and direct bearing upon mooted biological problems. This is the science of the *colloidal state*. The difficult abstractions and elaborate classificatory scheme, in terms of which the theory is now stated, will tend to be cleared up as our study of colloids comes definitely under the dominion of the general electro-molecular theory of matter. Intimate contact with the latter has already been established, indeed, through recent remarkable contributions by Langmuir,[8] dealing with the atomic constitution of solids and liquids. It is to colloidal chemistry that we must look for answers to the large majority of the fundamental problems of vital activity. These answers will be slow in appearing, however, if we refuse to look.

In fairness, it must of course be admitted that many biologists are keenly alive to the importance of the theory of matter, and especially of the theory of colloids, for the advancement of their science. However, possibly because the majority of these men are specialists in biochemistry, there seems to be a lack of coherent applications of mod-

[7] *Loc. cit.*, 41.

[8] Langmuir, I., "The Constitution and Fundamental Properties of Solids and Liquids," *Journal of the American Chemical Society* (1916), 38, 2221–2295; and other forthcoming papers in the same journal and in the *Physical Review*.

ern physico-chemical ideas to the problems of evolution and heredity, which make up the heart of the biological mystery.

It has for some years been my conviction that the conception of *enzyme action*, or of *specific catalysis*, provides a definite, general solution for all of the fundamental biological enigmas: the mysteries of the origin of living matter, of the source of variations, of the mechanism of heredity and ontogeny, and of general organic regulation.[9] In this conception I believe we can find a single, synthetic answer to many, if not all, of the broad, outstanding problems of theoretical biology. It is an answer, moreover, which links these great biological phenomena directly with molecular physics, and perfects the unity not alone of biology, but of the whole system of physical science, by suggesting that what we call life is fundamentally a product of catalytic laws acting in colloidal systems of matter throughout the long periods of geologic time. This view implies no absurd attempt to reduce every element of vital activity to enzyme action, but it does involve a reference of all such activity to some enzyme action, however distantly removed from present activity in time or space, as a necessary first cause. Catalysis is essentially a determinative relationship, and the *enzyme theory of life*, as a general biological hypothesis, would claim that all intra-vital or "hereditary" determination is, in the last analysis, catalytic.

The conception of enzyme action is, of course, one with which all biologists, including students of genetics, are extremely familiar.[10] Probably there is no student of morphogenesis who would not consider it absurd to deny that enzymes play a very important rôle in individual development. In a number of cases such participation has been clearly demonstrated by experiment, and the suggestion that the germ-cell contains "determiners" for

[9] See my two papers: "The Chemical Origin and Regulation of Life," *Monist* (1914), 22, 92–134; and "The Enzyme Theory of Life," *Cleveland Medical Journal* (1916), 15, 377 ff.

[10] On enzyme action in general, see Bayliss, W., "The Nature of Enzyme Action," 1914.

the production of enzymes, which, in turn, regulate certain aspects of the development, is a common one.[11] Several Mendelians have even hinted that the "unit characters" themselves are enzymes,[12] but so far as I am aware, no worker in genetics, with the exception of Goldschmidt, has regarded this conception as an important one.[13] Indeed, in the face of the nearly self-evident, they have turned away to vitalism and despair.

Consider, for example, the following quotation from Bateson.

> We must not lose sight of the fact that though the factors operate by the production of enzymes, of bodies on which these enzymes can act, and of intermediary substances necessary to complete the enzyme action, yet these bodies themselves can scarcely be genetic factors, but consequences of their existence. What are the factors themselves? Whence do they come? How do they become integral parts of the organism? Whence, for example, came the power which is present in a White Leghorn of destroying—probably reducing—the pigment in its feathers?[14]

It is my contention in this and previous papers that statements of this sort can hardly represent anything less than intellectual blindness. On the supposition that the actual Mendelian factors are enzymes, nearly all of these general difficulties instantly vanish, and I am not acquainted with any evidence which is inconsistent with this supposition.

III

Up to very recent times, although a great number of hypotheses to explain catalysis were in existence,[15] no

[11] See, for example, the following: Loeb, J., and Chamberlain, M. M., "An Attempt at a Physico-Chemical Explanation of Certain Groups of Fluctuating Variations," *Journal of Experimental Zoology* (1915), 19, 559–568. Moore, A. R., "On Mendelian Dominance," *Archiv für Entwicklungsmechanik* (1912), 34, 168–175. Riddle, O., "Our Knowledge of Melanin Color Formation and its Bearing on the Mendelian Description of Heredity," *Biological Bulletin* (1908), 16, 316 ff.

[12] See Bateson, "Mendel's Principles of Heredity," 1909, 268.

[13] The speedy publication of experimental results of great importance in this connection is promised by Goldschmidt. See above references.

[14] Bateson, "Problems of Genetics," 86.

[15] A comprehensive review of these theories and of the facts of catalysis and fermentation is given by Mellor, J. W., "Chemical Statics and Dynamics," 1914, 245–383.

completely satisfactory general theory of the process could be formulated. In this state of affairs, the use of the conception as a general explanatory agent in biology, could not be said to establish an unequivocal bond between biological regulation and the theory of matter. At the present day, however, it is possible to frame a hypothesis to account for catalytic action, which has general applicability and at the same time rests directly upon the ideas of modern molecular physics.

Ostwald defines a catalytic agent as "a substance which changes the velocity of a reaction without itself being changed by the process."[16] In the older terminology of the pioneer, Berzelius, it is "a substance which, merely by its presence and not through its affinity, has the power to render active affinities which are latent at ordinary temperatures."[17] According to Ostwald, catalytic power is a universal property of matter, for he says:

> There is probably no kind of chemical reaction which cannot be influenced catalytically, and there is no substance, element, or compound which can not act as a catalyzer.[18]

This being the case, it should often occur that a substance will catalyze a reaction which generates further quantities of the same substance, a process known as *autocatalysis*. Catalytic relationships may thus be classified into the autocatalytic and the *heterocatalytic*. I shall attempt to show that the former may be the more fundamental of the two relationships, and that reasons can be adduced for regarding autocatalytic power as a necessary property of every complex form of matter.

Perhaps the simplest illustration of a catalytic effect of any sort is that of the production of crystallization in a supersaturated solution of some substance by the introduction of a small crystal of the same substance. This of course has the form of an *auto*catalytic process. Although effects of this kind are included in Ostwald's classification of varieties of catalysis,[19] up to recent times

[16] Mellor, *loc. cit.*, 250.
[17] *Ibid.*, 246.
[18] *Ibid.*, 254.
[19] *Ibid.*, 255.

it might have been possible to raise a legitimate objection to the illustration on the ground that the induced change is not a chemical one. However, this objection is definitely disposed of by the recent work of the Braggs, and others, on the constitution of crystals,[20] which has shown that the unit of structure in solid bodies is usually the single atom, and not the molecule, since in crystals there is, as a rule, no exclusive arrangement of the atoms into molecular groups. The spacing of the atoms is such as to make it clear, moreover, that the forces which hold the total crystal system together are identical with those which we regard as underlying chemical affinity. In other words, in the crystal there is either no distinction between inter-atomic and inter-molecular forces (*i. e.*, between chemical affinity and cohesion), or else the entire crystal must be considered to be a huge polymeric molecule.[21] It is therefore perfectly legitimate to treat the process of crystallization as a chemical change, and to regard the initiation of this process under the conditions above described, as an example of autocatalysis, which may well be typical.

Although the results of crystal analysis indicate that no distinct molecules are to be found in the solid state, this is not true of the dissolved, or of the gaseous state. Moreover, on account of the fact that their component particles are held in place by forces of electrical attraction and repulsion, all molecules must possess their own fields of electrical force, and the field of any molecule must have a spatial form which is characteristic of that molecule. These field patterns will thus be different in the molecules of substances which differ chemically, and will be similar in molecules of the same or of an allied chemical substance.[22] The forces of cohesion in a crystal may be thought of as resulting from the fusion of a large number of these molecular fields into a continuous mosaic, and in such manner that their several axes are parallel.

[20] See Bragg, W. H., and W. L., "X-Rays and Crystal Structure," 1915.

[21] *Cf.* Langmuir, *loc. cit.*, 2221–2222.

[22] See Comstock and Troland, *loc. cit.*, 86–89.

However, such fusion can not fail to have an influence upon both the form and the strength of the fields in question, since it involves a redistribution of the atomic forces. This will take the form of an opening out, or expansion, which will necessarily reduce the coherence of the group of atoms originally forming the individual molecule. The degree of this "opening" of the field which occurs in crystallization must vary with the nature of the molecule, and is probably smaller for organic substances than it is for the majority of inorganic compounds.

The mechanism of the autocatalytic process of crystallization may be described somewhat as follows:

In a solution, or a gas, the molecules of the dissolved substance move about at random among the molecules of the solvent, and the orientation of the axes of their fields is entirely haphazard. However, as soon as a crystal of the solute is introduced, the field forces of the surface layer of atoms attract the dissolved molecules and at the same time tend to turn them on their axes so that, as they condense, they will fall into the pattern of the "space lattice" upon the plan of which the crystal is built.[23] As this is the most stable position which they can assume, they will tend to remain there and form a new surface layer of the crystal, to act in turn upon further molecules in the solution, until all of the surplus dissolved substance has been deposited.

The primary force bringing the molecules to the crystal face is of course not the surface field of attraction—surface tension field—but their temperature motion—or osmotic pressure. A similar force, of lower magnitude in the case of a supersaturated solution, is constantly disengaging molecules from the crystal and throwing them back into the solution. The action apparently ceases when the number of molecules deposited upon the crystal surface in unit time becomes reduced—owing to decreasing concentration—to an equality with the number leaving in the same interval.

[23] *Cf. ibid.*, 113.

The essential feature of the above described mechanism for the autocatalytic production of polymeric molecules may be illustrated to the eye by means of a model consisting of a board with a large number of small compass needles mounted upon it. If these needles are freed from the action of the terrestrial magnetic field and are then shaken into a random orientation, they may remain in this condition indefinitely. However, if a small number of adjacent needles be turned by some outside force so as to acquire a common direction, their combined magnetic fields will cause other neighboring needles to swing into line, so that the action must spread to all of the needles on the board. The field of an ideal compass needle has a simple bipolar pattern, and a symmetrical distribution of forces. In the cases of specific atoms and molecules, however, this is probably seldom true. Nevertheless, the general principles involved in their dynamic interaction would remain the same as those for the case of the compass needles.

It is clear that the explanation of autocatalysis above given accounts immediately only for the synthesis of polymeric molecules from individual units which are all alike. As a rule, chemical changes involve the interaction of different units, and it can easily be seen that the same general mechanism will apply to the catalysis of reactions of this sort as to that of simple crytallization. The principles involved in the process have been made especially clear in the recent articles of Langmuir.[24] Consider first a solution containing two kinds of molecules which can be deposited upon a crystal surface consisting of an orderly arrangement of these two molecular groupings in mosaic or lattice form. The second species of molecules may be considered, for example, to be those of the solvent, as in the case of "water of crystallization." There will be certain "elementary spaces"—as Langmuir calls them—upon the surface of the crystal, which will especially attract and orient the water molecules, and adjacent elementary spaces which will act in the same way upon the

[24] *Loc. cit.*, 2286–2292.

molecules of the solute. In this way the crystal or polymeric molecule will be built up out of two components by the simultaneous and parallel action of two initially combined species of molecular fields. This change is catalyzed by the crystal, and is an autocatalytic process involving the synthesis of two substances. It is clear that any number of substances may be influenced in this way by a similar, but more complex initial crystal form.

However, our explanation still remains somewhat special in its application, as in the majority of cases the products of catalysis do not adhere permanently to the catalytic surface. The extension of the explanation to cases of this sort is not difficult, since we have already seen that, even in the case of crystallization, the heat vibrations of the atoms are constantly throwing off molecular groups from the surface of the solid. As pointed out by Langmuir, the attraction between the surface and two molecular groups which have a strong affinity for each other may be less than the sum of the attractions of the surface for each of the groups, when separate.[25] This is due to the "closing up" or contraction of the fields of force of the groups as they come together. Hence combined groups of this sort will be more easily detached from the surface than will the uncombined groups, which will tend to be held in place until their mates fall into the right positions. The catalytic surface thus acts like an orienting sieve which on account of its special structure forces a chaotic crowd of individuals which come into contact with it, to fall into a special configuration. Many machines which accomplish exactly this effect are in use in the industries.

Thus far we have dealt only with the mechanism of autocatalysis. Heterocatalysis is probably to be regarded as an extension of the process of autocatalysis. It is obvious that exact similarity of the force patterns of the catalyzing and catalyzed systems is not essential. Indeed, the catalytic effect which is based upon direct similarity of structure between the two systems should be

[25] *Ibid.*, 2257, 2264–2266.

much weaker than that which accompanies certain types of structural *correspondence*, such as that existing between a body and its mirror-image, or between a lock and a key. Special structural relations of this sort probably exist between stereochemical isomers, between acids and bases, etc. It is easily conceivable that the patterns of certain surfaces may be capable of distorting other special configurations which come under their influence, so that they fall into new equilibrium figures, without these figures being of necessity identical with those of the catalytic system. The general principles of the action, however, remain the same.

Catalytic synthesis is a less common process in the laboratory than is destructive catalysis, but the laws of energy necessitate both effects, if either one is possible. Consequently the mechanism which we have described above must be an exactly reversible one, and must assist in the decomposition of molecular complexes as much as it aids in their synthesis. The deposition of the molecules to be decomposed, upon the catalytic surface would naturally follow the same principles as those stated for simple polymerization. In this state of deposition the field forces of the crystal surface would inevitably have a tendency to open up the field of the deposited molecule, thus rendering it more unstable than before, in which condition the temperature vibrations of the system could break it up more easily than in the undeposited state.

This weakening of the internal bonds of the molecule in the field of the catalytic surface corresponds with the weakening of forces of electrical attraction by increasing the "dielectric capacity" of the medium in which an electrical system is contained. It is the same action which permits water to dissociate neutral molecules into ions,[26] and is probably responsible for the high catalytic power of water, in general. However, in detail, the process must be a "personal" affair between individual water molecules and molecules of the dissolved substance, just as in the case of the crystal surface, since the ionizing effect

[26] *Cf.* Comstock and Troland, *loc. cit.*, 139–140.

of water does not appear to depend merely upon the chemical instability of the solute.

The increase in reaction velocity which characterizes catalysis is to be attributed to three more or less separable influences exerted by the catalytic surface, (1) the local increase in the concentrations of the reacting substances at the surface, (2) the impressment upon the attached molecules, of a relative orientation which is favorable to chemical union, or which in part constitutes such union, and (3) the spreading and weakening of the fields of force of the molecules, due to their interaction with the surface fields. The first factor, alone, would be of primary importance for the combination of free atoms —a relatively rare process—while the last factor, alone, would be responsible for the acceleration of simple decompositions. Reactions between two or more molecular *groups*, whether synthetic or metathetic, should be influenced by all three factors. Strutt[27] has shown that in certain typical chemical reactions, only one out of many millions of collisions between potentially reactable molecules results in chemical interaction. The active collisions probably coincide with the presence in the colliding system of favorable relative orientations and states of the molecular fields, which in the absence of a catalyzer depend upon chance, but which in the presence of a catalyzer are encouraged by the nature of the catalytic surface.

It is of course not possible in a paper of this sort to enter into the mathematics of the theory of catalysis which is outlined above.[29] Catalytic influence is obviously only one among many factors which affect a chemical reaction. Catalysis is possible only when the appropriate raw materials are provided, and when the energy relations of the system are such as to make the reaction thermodynamically conceivable. The heterocatalytic effect of a given substance may far outweigh its autocatalytic effect either

[27] Strutt, R. J., "Molecular Statistics of Some Chemical Actions," *Proceedings of the Royal Society* (1912), A, 87, 302–309.

[28] *Cf.* Mellor, *loc cit.*, 250–254. Also Bayliss, *loc. cit.*, 49–71.

[29] For a development of the mass action relationships involved, see Langmuir, *loc. cit.*, 2287 ff.

because the energy changes do not favor the latter, or because in a given system the raw material for the autocatalytic reaction is absent, while that for the heterocatalytic reaction is present in abundance.

However, the above considerations would lead us to believe that all substances should show some tendency to form polymeric molecules or crystals. This appears to conflict with the classical division of substances into crystalloids and colloids, but this division, like all others, can not be expected to stand unmodified by the modern analysis. There is plenty of evidence from direct observation that many colloidal particles are simply very small crystals.[30] On the other hand, the molecules of polymeric substances of high molecular weight, such as starch and certain proteins, are probably of the same order of magnitude as small colloidal particles. From the point of view of the theory of matter, there is no fundamental difference between the general plan of a starch molecule and that of a crystal of sugar, and it is highly probable that the distinction between colloids and crystalloids rests upon purely quantitative relations, respecting the size of the polymeric structures (crystals) produced under ordinary conditions.

Large crystals are formed easily by simple substances whose molecules have open fields of force or highly unsaturated attractions. Small crystals are characteristic of more complex substances, common among the compounds of carbon, having relatively closed fields. Large mosaics of such molecules become unwieldy and are easily disrupted by the temperature vibrations. They are also built up more slowly than are mosaics of molecules with open fields. The distinction between these two classes of molecules is of course merely quantitative; no type of molecule has a completely closed field, and on the other hand no substance is capable of forming indefinitely large crystals in a finite length of time. The atomic structure of the solid phase of a colloidal gel is probably analogous

[30] See Ostwald, Wo., "A Handbook of Colloid-Chemistry," English translation, 1915, 56–66.

to that of the mass of small crystals, compacted together, which always results from the rapid crystallization of a supersaturated solution of a substance like, *e. g.*, sodium thiosulphate.

It is evident, then, that the general theory of catalysis which has been outlined is applicable to *enzyme action*, which almost certainly depends upon the deposition, or *adsorption* of the reacting substances upon the surfaces of colloidal particles.[31] Such adsorption, the molecular mechanism of which has been made very clear by Langmuir,[32] will tend to be specific, and the more specific the more complex is the structure of the units making up the mosaic of the surface. Molecules the field patterns of which fit closely into the fields of the surface will tend to displace others having a cruder correspondence. This follows from either electro-dynamics or thermodynamics, and obviously coincides with Fischer's classical conception of the lock and key relation between enzyme and substrate.[33]

It will be perceived that our theory of the catalytic process is simply a refinement and extension of the classical theory of "intermediate compounds," which has been proven true in so many instances.[34] "Adsorption compounds," which play the principal rôle in enzyme action, do not differ dynamically from chemical compounds in general, since the forces causing adsorption are the same as those responsible for chemical union. Conversely, catalytic action in which the catalyst is in a molecular or unpolymerized state will not necessarily differ in its mechanism from that characteristic of enzymes or of metallic surfaces.

IV

The suggestion that the fundamental life-process of *growth* is the expression of an autocatalytic chemical re-

[31] See Bayliss, *loc. cit.*, 104–123.

[32] *Loc. cit.*, 2267–2278.

[33] See Mellor, *loc. cit.*, 363.

[34] *Cf.*, *e. g.*, Kendall, J., and Booge, J. E., "Studies on Catalysis. I. The Addition Compounds of Esters with Organic Acids." *Journal of the American Chemical Society* (1916), 38, 1712–1736.

action has been made independently by a number of investigators.[35] It will be perceived that on the basis of the foregoing theory of autocatalysis, this suggestion becomes closely allied to the familiar and ancient comparison of vital growth to the growth of a crystal. The customary objection to this comparison, viz., that a crystal grows by accretion whereas protoplasm increases by intussusception, loses its force as soon as we regard living matter as a complex mixture of substances suspended by colloidal subdivision in water, since there is no evidence that the individual colloidal particles do not grow by accretion. On the contrary, it is almost inconceivable that these bodies, which are the real chemical units of protoplasm, should grow in any other way. The growth of a system like a cell could be regarded as the resultant effect of a very large number of component growths, each governed by its specific autocatalytic mechanism. It has been shown by T. B. Robertson[36] that growth curves, with respect to the time, actually do coincide in general form with the curve characteristic of an autocatalytic reaction.

A multitude of observations substantiate the belief that the internal determination of cell-life rests primarily with the nucleus,[37] or with the chromatin substance of the cell, when no well-defined nucleus is present. Even in the highly organized cell, this substance can be seen to possess a mosaic structure, and it can be shown that for a given species this structure is sensibly constant,[38] so that it is necessary to suppose that a reduplication of chromatin units occurs with each cell-division. This process of reduplication is apparently made visible to us in mitosis.

35 See, for example, Ostwald, W., "Ueber die zeitlichen Eigenschaften der Entwicklungsvorgänge." Vorträge und Aufsätze über Entwicklungsmechanik des Organismus, herausgegeben von W. Roux (1908), Heft 5, Leipzig.

36 Robertson, T. B., "On the Normal Rate of Growth of an Individual and its Biochemical Significance," *Archiv für Entwicklungsmechanik* (1908), 25, 581–615; and subsequent articles in the same journal.

37 See Wilson, E. B., "The Cell in Development and Inheritance," second edition, revised (1911), 30–31, 341–354.

38 *Cf.* Boveri's *Individualitäts Hypothese* and "law of proportional nuclear growth."

The simplest hypothesis to account for such reproduction lies in the supposition that each unit can give rise to another unit substantially identical with itself.

The Weismannian theory of the constitution of the germ plasm,[39] which is typical of the so-called "corpuscular theories" of the life-process or of heredity, also demands the existence of vital elements, each possessing the power of reproduction *ad infinitum*. The general conceptions of this theory appear to find verification, first, in the facts already mentioned, and second, in the discoveries of Mendelism. The recent work of Morgan and his collaborators,[40] moreover, reveals clearly the intimate connection which exists between the corpuscular "unit characters" of Mendelian heredity and the histological units present in the chromosomes. Consequently, it would seem to be a fairly safe generalization, or at least an extremely probable hypothesis, which states that the distinctive properties of cells, tissues, and species are primarily determined by the nature of systems of colloidal particles contained in cell-nuclei and, originally, in some germ-cell nucleus.

However, in spite of the seeming strength of the evidence, some biologists are of the opinion that such a view as this must be rejected because it paralyzes thought. Consider, for example, the following quotation from Child.[41]

> It is scarcely necessary to call attention to the fact that these [corpuscular] theories do not help us in any way to solve any of the fundamental problems of biology; they merely serve to place these problems beyond the reach of scientific investigation. The hypothetical units are themselves organisms with all the essential characteristics of the organisms that we know; they possess a definite constitution, they grow at the expense of nutritive material, they reproduce their kind. In other words, the problems of development, growth, reproduction, and inheritance exist for each of them, and the assumption of their existence brings us not a step nearer the solution of any of these problems. These theories are nothing more nor less than translations of the phenomena of life as we know them into terms of the activity of

[39] Weismann, A., "The Germ Plasm," English translation, 1893.

[40] *Loc. cit.*

[41] Child, C. M., "Senescence and Rejuvenescence," 1915, 11–12.

multitudes of invisible hypothetical organisms, and therefore contribute nothing in the way of real advance. No valid evidence for the existence of these units exists, but if their existence were to be demonstrated we might well despair of gaining any actual knowledge of life.

We have in this passage a clear statement of the essentiality of growth, as self-reduplication of specific substance, in the life-process. Consideration of Child's remarks will show that the difficulties which he raises are almost completely dissolved as soon as we postulate for the biological corpuscles the power of autocatalysis. In the light of our previous discussion, it can not be claimed that this is purely a verbal solution of the problem, as we have advanced definite reasons for believing that autocatalytic activity is a property of all chemical substances whatsoever, given the appropriate chemical environment. Since the environment of the chromatin particles has been made to order by evolution, the force of Child's criticisms would seem to be nil. Moreover, he certainly underestimates the importance of the facts which point to an actual corpuscular determination of vital functions.

In view of this, it would appear advisable to accept the *Elementarorganismen*[42] as if they were clearly established facts, and proceed to consider what further light can be thrown upon biological problems by the conception of specific catalysis.

It is well known that in many cases, at least, the nucleus controls cell activity by liberating enzymes,[43] and the mass activity of cells in the form of specific tissues has been satisfactorily proven to depend, so far as it is directly chemical, upon the presence in these tissues of specific enzymes. In the field of general adult metabolism the determinative importance of catalysis would appear to be no longer a matter of debate. Analogy would lead us to believe that the same principle is of prime importance in the metabolism of development.

In laying emphasis upon the cardinal importance of the

[42] See Brücke, *Sitzungsbericht, Akademie der Wissenschaften*, Wien, (1861), 44, (2), 381–406.

[43] See Mann, G., "The Chemistry of the Proteids," 1906, 454 ff. Also, Loeb, J., "The Dynamics of Living Matter," 1906, 7–29.

enzyme for organic regulation we must of course recognize that the exact effects produced by catalysis depend, at all stages of development, upon the manner of its cooperation with other physical principles, which may involve the functioning of molar structures already present. However, as we retrace the course of ontogeny and of the evolution of any specific germ-cell, we should find that the number and importance of such structures decrease, although the construction of any given tissue-form always depends upon the action of specific enzymes in conjunction with preëxisting tissue structures. It is not to be doubted for an instant that important preëstablished structures exist even in germ-cells, and enter into the determination of their activity. It is therefore unfair to demand a catalytic explanation of such a complex process as karyokinesis, which shall not take into consideration the history or evolution of the cell.

The task of elucidating the exact mechanism by means of which vital regulation is maintained, and especially of showing how, in accordance with recognized principles of physics, a complex of specific, autocatalytic, colloidal particles in the germ-cell can engineer the construction of a vertebrate organism, is truly so formidable that it is unkind for the vitalist arbitrarily to deny us the use of any of these recognized principles. For example, we must be permitted to suppose that a large number of variables can unite in the production of a single effect. The greater part of the modern vitalistic worry over "organization" and vital "equilibrium"[44] appears to depend upon a tacit assumption, either that physical laws are not reliable, or that it is impossible for a number of variables to control simultaneously a single process. Both of these assumptions are self-evidently counter to the most fundamental presuppositions of physical analysis.

Although the fundamental life-property of the chromatin units is that of autocatalysis, it is necessary and legitimate to suppose that the majority of them sustain specific heterocatalytic relationships to reactions oc-

[44] Consider, for example, Haldane, *loc. cit.*

curring in living matter. This is because nuclear material makes up a relatively small percentage of protoplasm, and because the reactions governed by enzymes are ordinarily heterocatalytic.

It is a remarkable fact that the chemistry of the cell-nucleus has reached a stage of advancement superior to that attained by the chemistry of the cytoplasm. It appears that the essential constituent of chromatin is a substance called nuclein, which is composed of a basic, protein factor and nucleic acid. The facts indicate that the acid factor is the permanent and essential component of the nucleus, and organic chemical analysis seems to prove that only one kind of nucleic acid exists in animal tissues, although a different variety is to be found in the cells of plants.[45] If, as now seems probable, the genetic enzymes must be identified with the nucleic acids, we shall be forced to suppose that these substances, although homogeneous—in animal or plant—from the point of view of ordinary chemical analysis, are actually built up in the living chromatin, into highly differentiated colloidal, and colloidal-molar, structures. The apparent homogeneity results from the fact that ordinary chemical analysis provides us only with the *statistics of the fundamental radicles* which are involved.

To some minds, the idea that a portion of matter as small as a germ-cell can contain sufficient catalytic substance to control the destinies of a complex organism, seems hardly plausible. However, considering the slowness of such processes as growth, it is clear that the quantity of catalyzer required will usually be smaller than that used in laboratory experiments; and it is a truism in chemistry that radical alterations of reaction velocities can be caused by the presence of almost infinitesimal amounts of catalytic material.[46] From the nature of the process, it is evident that only a *few molecules* of substance will be required to furnish the basis for an *auto*-catalytic reaction which may eventually result in the pro-

[45] See Jones, W., "The Nucleic Acids," 1914.

[46] See Mellor, *loc. cit.*, 248–249.

duction of any desired amount of this substance; and a simple calculation shows that the chromatin of the human zygote has sufficient volume to contain about one quadrillion (10^{15}) molecules the size of that of oxygen.[47]

In order that the enzymes of the germ-cell should be able to determine the form of the mature organism, they must have the power to govern (1) the physical and chemical properties of specific tissue material, (2) the position of specific tissues, (3) the size of these tissues and (4) their form. Since the physical properties of any piece of matter depend upon its chemical constitution, and since any chemical change can be regulated by catalysis, the mere presence of a specific catalyzer in a favorable mixture is sufficient to determine the production of matter of any possible variety, in any possible amount. It is always necessary to assume that the history of an organic system is such as to have provided it with the raw materials necessary to its activities. If this is not the case, the system naturally perishes of "starvation."

The most primitive form of cell-division involves nothing more than reduplication, and this is the law of multiplication of the germ-plasm. Driesch[48] argues that to explain the reproduction of a nuclear "machine" which determines development, we must postulate another machine to carry out the operation, and so on *ad infinitum*. The nature of the autocatalytic process, however, shows that this conclusion is in error, since pure autocatalysis would tend to bring about an exact qualitative reproduction of any given plane or linear mosaic of specific units. In a nutritive medium such a mosaic would tend to grow in all of its parts by the deposition of similar substance. Primitive nuclear division (as, *e. g.*, in the Protista) may depend solely upon the physical instability of colloidal particles greater than a certain size, but it can hardly be

[47] This calculation is based on the following assumptions: (1) that the diameter of the germ-cell nucleus is .05 mm., and (2) that the molecules fill only one-sixth of the total volume of the nucleus.

[48] Driesch, H., "The Science and Philosophy of the Organism," 1908, 2, 341.

doubted that the complex mechanism of mitosis rests upon definite structural machinery, established by long periods of evolution.

In order to account for the differentiation of cell-nature which occurs in ontology, Weismann was led to assume a thoroughgoing differential segregation of the biophores of the original germ-cell in the course of embryological development; in other words, he supposed that in this process the rule of nuclear division is differentiation and not reduplication. This assumption, although undoubtedly a partial truth, is neither necessary nor in harmony with general biological probabilities, in the form in which it was made by Weismann.[49] Consequently the difficulties into which it has led his general theory, can be regarded as without important bearing upon the acceptability of corpuscular hypotheses at large.

Since reduplicating division is the established rule among unicellular organisms—which must have had a long evolutionary history—we should expect this rule to be conserved as far as possible in multicellular evolution. According to the general law of recapitulation, this should be especially true for the primary stages of ontogeny, for which Driesch's principle of the "equipotential system" appears often to hold. The blastula may well be simply an undifferentiated mass of germ-cells, analogous to a homogeneous colony of unicellular forms. Rudimentary differentiation may be brought about and determined by specific enzyme constitution, without differential partitionment of enzymes in segmentation, since the forces acting upon any cell must depend upon its *position* in the mass, and the activation or inhibition of a given enzyme may be conditioned by the presence of definite stimuli in definite intensities. This being the case, any cell could assume germinal characters if isolated from the total mass.[50]

[49] Weismann's theory, it must be recognized, assumes "doubling division" for the early stages of segmentation, a law which continues to hold for the "germ-tracks."

[50] *Cf.* Hertwig, O., "Evolution or Epigenesis," English translation, 1896.

However, the facts of "crossing over" observed in recent studies on the relation between Mendelian characters and chromosome constitution[51] show that the latter is not inviolate, even in purely germinal segmentation. Since the power possessed by cells to assume germinal character, even to the limited degree of being able to regenerate a single organ or tissue, seems to vary in inverse proportion to the degree of specialization of the cells, it is reasonable to suppose that Weismann's principle of differentiating division actually does operate in the higher stages of development. However, at no stage is it the only mechanism of differentiation, and it certainly is not the primitive means.

It is possible that cancer represents a return of tissue cells to a germinal or semi-germinal stage, due to the failure of a "stimulus of differentiation" to remain effective.

The control by the *genetic enzymes* of the position, size and form of specific tissues must involve, first, a quantitative regulation of the process of differentiation, which can be effected by the establishment of definite relations between the chemical constants of the catalytic reactions and the conditions under which the course of development necessarily places them; and, second, control of the planes of segmentation of the cells. To attempt a specification of the exact process by which this latter factor can be governed by the chemical constitution of the cell-nucleus, lies beyond the scope of the present paper, but it should be pointed out that in the last analysis chemical constitution means nothing but a definite spatial arrangement of electrical forces, so that there is nothing paradoxical in the determination of "pure form" by chemical agents.

As is evident in the quotations made above from Bateson, the dominant problem in the modern discussion of evolution is that of *the origin of variations*. It is the failure of Neo-Darwinians to explain the appearance of variations, and especially of new unit characters, which has led such writers as Driesch, Korschinsky and Wolff

[51] See Morgan, *loc. cit.*,

to speak of the "episode of Darwinism" and of *Das Sterbelager des Darwinismus*.[52] The enzyme theory of vital determination brings new life to the doctrine of evolution by accidental variation and natural selection, first, by showing that all fundamental variations should be discontinuous, or heterogenetic, as demanded by the mutation theory of De Vries, and second by revealing the exact mechanism of the production of these variations. The discontinuity follows from the existence of qualitative gaps between all specific chemical substances, such as those making up the system of genetic enzymes. The mechanism of production of variations is simply that of the initial production of any new chemical individual, *i. e.*, the fortuitous encounter of the appropriate molecules with the right relative orientations and at the correct speeds (*vide supra*). The "chance" nature of variation thus is made to depend upon that "molecular chaos" which is so very familiar to all physicists, but the implications of which for biology have thus far been largely neglected.

A moment's thought will show that, on the basis of the enzyme theory, variation should be *additive*, since an autocatalytic individual, once established, will tend automatically to maintain itself. The complete elimination of such individuals will occur only through the destruction of the entire germinal mosaic of which they form a part, an effect accomplished by natural selection unless the new enzyme is in harmony with functions which preserve the organism. It is very important to bear in mind that the catalytic complex which is supposed to underlie organic development and regulation has been determined in its nature by excessively exhaustive practical tests and, as a complex, by nothing else. It is therefore not surprising that the practical delicacy of the regulation which it actually subserves should be very great.

In other papers, I have discussed somewhat in detail the bearing of the enzyme theory upon the problem of the *origin of life*. On the basis provided by this theory, the

[52] See Kellogg, V. L., "Darwinism To-day," 1907.

origin of life can not be regarded as a catastrophic event; life depends upon an organized complex of selected catalytic material, and hence *some* life originates with each new, successful mutation. Of course, if we trace the process of the evolution of any given species back sufficiently far, we must eventually come to the *first mutation*, which would consist in the molecular production of an autocatalytic particle sustaining relations with its environment such as to make possible its continued growth and reproduction. I have used the name *protase* to stand for the "first enzyme" of the archebiotic process, but there is no particular reason for supposing that there was only one enzyme to which this name could apply.

There is considerable evidence that free autocatalytic enzymes exist in our biological universe even at the present day. Such an hypothesis would serve to account for the specific contagious diseases, such as measles, rabies, and smallpox, which have been demonstrated to possess "filterable viruses." The so-called Chlamydozoa probably fall in this class.

That the Chlamydozoa consist of free chromatin material is suggested by the late Professor Minchin, in his admirable paper on the evolution of the cell,[53] with the main outlines of which the enzyme theory would entirely agree. The single cell, and so-called simple protoplasm, must be regarded as the products of a detailed process of evolution, and hence can not form the ultimate explanatory units in biology. Next to the free autocatalytic particle, the simplest typical life-structure would consist of a single particle of this sort surrounded by an envelope of semi-liquid and chemically homogeneous substance with which it sustains a heterocatalytic relationship. The most primitive substance of this kind might be called *eoplasm*, to distinguish it from complex protoplasm, and the physical system made up of protase and eoplasm would represent a living cell in its most reduced form.

Minchin says, in the article referred to:[54]

[53] Minchin, E. A., "The Evolution of the Cell," AMERICAN NATURALIST (1916), 50, 5–39, 106–119.

[54] *Loc. cit.*, 35–36.

The biochemist renders inestimable services in elucidating the chemical mechanisms of living organisms but the problem of individuality and specific behavior, as manifested by living things, is beyond the scope of his science, at least at present. Such problems are essentially of distinctive vital nature and their treatment can not be brought satisfactorily into relation at the present time with the physico-chemical interactions of the substances composing the living body. It may be that this is but a temporary limitation of human knowledge prevailing in a certain historical epoch, and that in the future the chemist will be able to correlate the individuality of living beings with their chemico-physical properties, and so explain to us how living beings first came into existence; how, that is to say, a combination of chemical substances, each owing its characteristic properties to a definite molecular composition, can produce a living individual in which specific properties are associated with matter in a state of flux.

It is my contention that the enzyme theory of life satisfactorily meets these general requirements.

V

To arrive at a proper estimate of the importance of a general theory such as the one discussed in the present paper, necessitates considerable reflection. The path of scientific progress is beset by the pitfalls of conservative empiricism, on the one hand, and by those of radical speculation, on the other. To the radicals the enzyme theory presents an aspect of *a priori* self-evidence; to the conservatives it seems to be a vague generalization with no particular or specific facts to support it, approximately on the same plane as the statement that "life is motion," which Driesch says is about as useful as the proposition that "Kant was a vertebrate." Regarding the general enzyme theory, the following opinion has been expressed to me privately by an eminent zoologist.

> The idea . . . is a perfectly familiar one. The trouble comes when we attempt to make a specific application of this idea to a concrete problem, which is what science demands if a pure speculation is to become a valuable working hypothesis. For instance, how an autocatalytic molecule could produce the phenomena seen in the division of the chromosomes in the cell is by no means clear; nor is it clear why such molecules brought together during fertilization separate from each other at the maturation division. It is these specific questions that must first be answered, I think, before we can make much advance in

regard to the nature of the phenomena. . . . Of course, I realize that general ideas are always important for the development of science, only I think they should be advanced with caution and all attempts to make them appear as specific explanations should be avoided.

There are a number of principles of scientific methodology which have a bearing upon criticisms of this sort. In the first place such a statement as that "life is motion" would have value at a time when the connection between these two ideas had not been noted, or had been underemphasized to such an extent that eminent scientists were bemoaning the inability of the human mind to account for properties of life which the most superficial examination would show to be identical with those of motion. From my own point of view the proposition that "life is determined by specific catalysis" appears to be somewhat of the nature of a truism, indeed so much so that even if we had no direct evidence for the existence of enzyme action, we should be forced to invent the conception to account for the most general properties of living systems. It seems to me that this is exactly what a considerable number of biologists actually have done, and the only important error in their thinking lies in the application to the concept of such names as "biophore," "determinant," "unit character," "formative factor," "*Elementarorganism*," "*élan vital*," or "entelechy." This error, however, is fatal to progress, as it multiplies terminology and delays the synthesis of actual ideas which is the goal of scientific endeavor.

Furthermore, it is not true that the establishment of a general principle necessitates an examination of all of the concrete details of specific systems. If this were so, none of the fundamental laws of mechanics, such as the first and second laws of energetics or Hamilton's principle, would possess any rigidity, since they are derived from a study of what may be called the "entrance and exit" properties of mechanical systems, without reference to their exact contents. It is to be admitted, of course, that we can not rest content with this kind of knowledge, and that principles of this sort receive complete elucida-

tion only when the details of all systems are made clear, but the security of the principles themselves is affected scarcely at all by this analysis.

I do not claim that the enzyme theory of life possesses a general basis as adequate, for example, as that of the principle of least action. I do claim, however, that this is because the latter can be stated in terms of an exact mathematical formula, whereas the enzyme theory has to be given a qualitative description. The enzyme doctrine is supported at the present time by a considerable number of specific facts of cell chemistry, but it possesses a far more substantial bulwark in the general facts of vital function. Shall we deny that these facts are adequately established, or that they are important, or that they merit explanation? Shall we reject a definite physico-chemical conception which at one stroke explains the majority of the mass relationships of living matter, on the ground that the details of some special life-processes have not yet been described in terms of this conception? Or is it preferable to preserve the inexplication of these same generalities to furnish a basis for vitalism?

There are an indefinitely large number of ways in which the principle of the conservation of energy can be exemplified in special pieces of machinery, and there are just as many ways in which the principle of specific catalysis can operate. Instead of holding the energy principle in abeyance until we have seen how the action of a special mechanical system can be explained in terms of it, we usually assume it to be true, and shortly find the action in question very easy to understand. This would seem to be the only feasible method for employing any theoretical proposition, even if it is merely a novel working hypothesis. The trouble which arises in the attempt to apply the enzyme theory to specific problems is a normal result of the inertia of the human imagination, which does not immediately outline a plan for a machine to accomplish a definite purpose, even when it is provided with all of the principles of mechanics. Surely, however, the plan can never be developed if such principles are neglected.

MUTATION IN DIDINIUM NASUTUM

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THE origin of heritable variations or mutations constitutes one of the most fundamental problems of biology. It has long since been recognized that evolution depends upon such variations, and they have consequently been extensively studied by a considerable number of investigators, *e. g.*, Darwin, DeVries, Bateson, Kammerer, Tower, Stockard, MacDougal, Jennings, Morgan, *et al.* These studies have resulted in the accumulation of a mass of facts of great importance, but the nature of the origin of the variations in question is still shrouded in mystery.

In a series of experiments on the effect of conjugation and encystment in *Didinium*, extending from April, 1910, to May, 1914, there suddenly appeared, in the latter part of July, 1912, a marked difference in the rate of fission in the progeny of a single individual. This difference appears to have been permanent, as the results presented herewith indicate. And it seems to show, in opposition to the conclusions reached by a considerable number of investigators, that variations in organisms reproducing asexually are at times heritable.

The difference in rate of fission mentioned was discovered in a group of five pure lines, all of which had been carried from the beginning of the experiment. These lines all originated from the same individual, and before the mutation occurred they had produced, without conjugating, an average of 721 generations; and without encysting, 197 generations. Throughout this entire period there was remarkably little difference in the rate of fission in the five lines. The total number of fissions produced by these lines during the 40 days immediately preceding the appearance of the mutation was respectively 164, 171,

168, 166, and 168. Thus it is obvious that in the ancestors of the mutants nothing in the nature of mutations in the rate of fission had occurred for many generations. This indicates that mutations do not ordinarily occur in asexual reproduction.

During the period of 40 days mentioned above, ending July 10, all of the lines were in excellent condition and not a single individual died. On July 12, however, one line died out and on the 14th three more died, leaving but one line. For several days preceding the temperature was very high. It was recorded twice daily and these records show that it reached a maximum on July 9, when it was 28.5° at 7:30 A.M. and 31° at 6 P.M. It was, however, high continuously from the 4th on, and during this period reproduction was exceedingly rapid, as Table I indicates. It was at the close of this period of rapid multiplication that the four lines mentioned above died out and it is probable that this extraordinary environmental condition had much to do with the nature of the variations in the progeny of the remaining line, although similar variations did not occur in four other groups of lines that were running parallel with the one under consideration.

From the remaining line mentioned above five new lines were started on July 15. For the first five days, the rate of fission in these lines was nearly the same, the total number per line being 26, 27, 28, 26 and 28, respectively. During the next five days the difference became somewhat greater, the total number of fissions per line being 21, 19, 18, 17 and 17, respectively. On the day following this period, the line which had produced 19 fissions died out and was replaced by a new line from the one which had produced 21. There were thus two lines having more rapidly, and three lines having less rapidly, dividing ancestors, all, however, originating from the same individual. On October 14 the lines in both groups were increased to five and thus they were continued until the close of the experiment, which extended through 315 days. The individuals in all of the lines in each group repro-

TABLE I

THE RELATION BETWEEN THE FISSION-RATES OF TWO STRAINS OR GROUPS OF LINES ISOLATED FROM THE PROGENY OF A SINGLE DIDINIUM

June 1, 1912 to May 27, 1913

Each column under the brackets represents a line and each number in the column the total number of fissions for five days; *d* indicates that the line died out; *c*, that it encysted; the brackets show the ancestry of the new lines established. The ancestors of the first five lines had passed through 553 generations without conjugation when these lines were started. Not all the lines that died out are indicated in the table. Whenever a line died out more than once in a five-day period, as sometimes happened, it is recorded only once.

Pure Line with 553 ± Generations without Conjugation					Average Total No. Fissions for Five-day Periods	
					First Group of Lines	Second Group of Lines
23	23	22	21	22		
14	15	14	15	15		
17	18	18	17	17		
18	19	18	18	21		
20	21	21	21	20		
23	22	22	22	21		
21	22	21	21	23		
28	31	32	31	29		
Total 164	171	168	166	168		
30	*d*	*d*	*d*	*d*		
26	27	28	26	28	26½	27⅓
21	19	18	17	17	20	17⅓
22	*d*	22	*d*	*d*	22	22
17	18	15	15	15	17½	15
20	20	15	16	16	20	15⅔
d	26	20	22	21	26	21
24	24	17	20	19	24	18⅔
25	26	18	24	19	25½	20⅓
20	21	13	18	15	20½	15⅓
c	19	15	16	15	19	15⅛
26	25	20	22	21	25½	21
25	24	19	19	20	24½	19⅛
17	17	14	15	14	17	14⅓
15	16	12	12	11	15½	11⅔
d	13	9	9	9	11½	9
7	7	3	5	3	7	3⅔
15	16	12	11	9	15½	10⅔
d	18	13	13	15	18	13⅔
8	8	9	10	8	6	7	5	8⅔	6
13	12	12	12	*c*	8	9	9	12⅓	8⅓

TABLE I (*continued*)

Pure Line with 553 ± Generations without Conjugation										Average Total No. Fissions for Five-day Periods	
										First Group of Lines	Second Group of Lines
d	10	11	9	*c*	8		8		7	10	$7\frac{2}{3}$
c	12	12	12	12	9		9		*d*	12	9
12	12	13	13	13	*d*	8	8	9	9	$12\frac{3}{5}$	$8\frac{1}{2}$
12	11	11	10	11	9	8	8	8	7	11	8
9	10	10	10	10	*d*	5	6	7	6	$9\frac{4}{5}$	6
9	10	10	*d*	11	7	5	6	6	*d*	10	6
10	10	*d*	8	9	5	3	5	*d*	5	$9\frac{1}{4}$	$4\frac{1}{2}$
9	9	9	9	9	5	*d*	5	*d*	6	9	$5\frac{1}{3}$
10	10	10	*d*	8	7	5	7	6	6	$9\frac{1}{2}$	$6\frac{1}{3}$
10	*c*	*d*	*d*	9	6	7	7	7	7	$9\frac{1}{2}$	$6\frac{4}{5}$
10	11	12	11	10	8	9	8	8	8	$10\frac{4}{5}$	$8\frac{1}{5}$
11	*c*	13	*c*	13	*c*	7	8	*d*	5	$12\frac{1}{3}$	$6\frac{4}{5}$
11	12	12	12	12	8	*c*	6	8	4	$11\frac{4}{5}$	$6\frac{1}{2}$
d	13	*d*	13	12	9	6	9	8	8	$12\frac{4}{5}$	8
d	14	14	14	14	10	12	10	10	10	14	$10\frac{2}{5}$
15	*d*	13	14	14	10	10	9	10	10	14	$9\frac{4}{5}$
12	12	12	13	12	9	8	8	8	8	$12\frac{1}{5}$	$8\frac{1}{5}$
13	13	13	15	14	10	10	9	10	10	$13\frac{3}{4}$	$9\frac{4}{5}$
12	11	11	*c*	12	9	9	8	9	8	$11\frac{1}{2}$	$8\frac{3}{5}$
10	10	10	11	10	8	9	7	8	6	$10\frac{1}{5}$	$7\frac{3}{5}$
11	11	11	11	11	8	8	8	8	7	11	$7\frac{4}{5}$
10	10	11	10	10	5	7	6	6	*d*	$10\frac{1}{5}$	6
8	11	11	11	10	8	8	6	7	8	$10\frac{1}{5}$	$7\frac{2}{5}$
10	13	12	11	12	9	11	8	8	8	$11\frac{4}{5}$	$8\frac{4}{5}$
11	14	*d*	*d*	*d*	10	6	11	11	10	$12\frac{1}{2}$	$9\frac{3}{5}$
10	10	*d*	12	9	9	7	*d*	8	*d*	$10\frac{1}{4}$	8
7	8	9	9	8	7	7	7	7	7	$8\frac{1}{5}$	7
9	13	12	12	*d*	10	8	9	9	9	$11\frac{1}{2}$	9
7	12	13	13	11	10	8	8	9	8	$11\frac{1}{5}$	$8\frac{3}{5}$
10	14	12	12	14	9	8	9	9	10	$12\frac{2}{5}$	9
8	12	12	11	12	9	7	8	9	7	11	8
d	9	8	9	9	6	*d*	*d*	6	6	$8\frac{4}{4}$	6
10	11	*d*	11	11	9	7	6	*d*	6	$10\frac{1}{2}$	7
8	8	8	6	*d*	3	7	7	6	6	$7\frac{1}{4}$	$5\frac{4}{5}$
9	9	7	*d*	*d*	7	*d*	7	7	7	$8\frac{1}{3}$	7

TABLE I (*concluded*)

Pure Line with 553 ± Generations without Conjugation										Average Total No. Fissions for Five-day Periods	
										First Group of Lines	Second Group of Lines
9	9	8	7	7	6	6	7	5	5	8	$5\frac{4}{5}$
12	*d*	11	12	13	9	9	8	*d*	7	12	$8\frac{1}{4}$
7	9	10	9	9	6	5	3	*d*	7	$8\frac{1}{5}$	$5\frac{1}{4}$
16	11	13	14	14	*d*	10	7	*d*	9	$13\frac{3}{5}$	$8\frac{2}{3}$
d	7	7	8	6	4	3	5	4	4	7	4
10	9	9	9	6	7	3	4	4	4	$8\frac{3}{5}$	$4\frac{2}{5}$
10	8	11	7	9	8	7	8	7	*d*	9	$7\frac{1}{2}$
d	8	10	*d*	10	6	*d*	8	5	*d*	$9\frac{1}{3}$	$6\frac{1}{3}$
Total average generations per line in 315 days										838 ±	634 ±
Number of lines died out										30	33
Number of lines encysted										8	3
Number of times conjugation occurred before transfer was made										2	0

duced at practically the same rate, but those in the former, considerably more rapidly than those in the latter. During the 315 days each line in the one group produced approximately 838 generations, $2\frac{2}{3}$ per day, and each line in the other group approximately 634 generations, 2 per day.

The averages for the five lines in each group for five-day periods are presented in Table I and plotted in Fig. 1. By referring to this table and the figure it will be seen that the difference in rate of fission in the two groups remained fairly constant throughout the entire 315 days, and that in both groups the rate was high in July and August, 1912, after which it decreased considerably and then remained fairly constant.

The fluctuations in rate of fission were closely associated with variations in temperature. This was true for twenty-four-hour periods as well as for the five-day periods given in the table. During July and August, when the fission rate was high, the temperature was in general much higher than it was during the rest of the time, when the fission rate was relatively low. At the

FIG. 1. Curves representing the fission rates in two groups of lines of *Didinium* originating from a single individual 721 ± generations after conjugation and 197 ± generations after encystment. Each point in the curves was obtained by averaging the rate of fission of all of the lines in each group for five successive days. Ordinates equal average daily rate of fission, abscissas equal days from the beginning of the experiment.

Note that the difference in the rate of fission in the two groups is essentially the same during the entire 315 days.

close of the experiment, however, the rate of fission was not as high as the temperature at this time would lead one to expect. The didinia in both groups appeared to be in poor condition. There were numerous very small individuals produced and an unusually large number of monsters. Conjugation was prevalent, but it was almost impossible to induce encystment. The death-rate was, however, not abnormally high. Whether or not the lines would have recovered from this depression if the experiment had been continued, is a question which can not be definitely answered.

Before the experiment was closed some cysts were secured in both groups. These were kept in a damp chamber as usual until the following year. Then they were put into culture fluids of various sorts containing paramecia; but only a few developed, all of which belonged to the more rapidly dividing lines. From these, five new lines were started and carried on for 40 days. During all this time the condition of the individuals was much as it had been immediately before encystment.

Throughout the entire experiment the didinia were cultivated in rectangular watch-glasses having a depression with a curved bottom. These dishes were piled one upon the other and kept in a damp chamber. All of the didinia were fed with paramecia from the same cultures. At each feeding an equal amount of solution was taken from two of the most vigorous of four pint cultures which were continuously kept in as flourishing conditions as possible by adding fresh water and a little timothy hay from time to time. The two equal quantities of solution were then thoroughly mixed and two drops of this mixture containing numerous paramecia were put into each of as many watch-glasses as there were didinia cultures. One drop of solution containing one didinium was then taken from each of the didinia cultures and added to each of the watch-glasses containing the paramecia. The remaining didinia, after recording the number of generations produced, were destroyed or used in studying conjugation

and encystment. During the coldest weather it was sufficient to transfer every other day, but during the warmest weather it was found advantageous to transfer twice a day. Nothing was sterilized in these experiments, but the same pipet was used in all transfers and the watch-glasses not in use were exposed to the air and allowed to dry. Moreover, from time to time the didinia in each line in either group were transferred directly to the watch-glasses from which the didinia of the other group had just been taken. In these dishes there always remained considerable solution, in some instances a drop or more. Furthermore, in a few cases didinia from the more rapidly dividing lines were transferred directly without the addition of fresh food to dishes in which more slowly dividing lines had died or from which all of the didinia had been removed.

Such treatment had no appreciable effect on the relative rate of fission in the two races. It is obviously evident, therefore, that the difference in the rate observed was not due to difference in the bacterial contents of the solution if there really was any such difference, nor was it dependent upon selection, natural or otherwise, for members of the more rapidly dividing pairs were always transferred in all lines. And the number of lines lost by death and encystment was essentially the same in both. In the one 30 were lost by death and 8 by encystment, in the other 33 by death and 3 by encystment. Assuming that the weaker lines died out in every case, it is evident that in this respect both races were subjected to practically the same sort of selection. And since all of the cultures were subjected to the same conditions otherwise, it is clear that the difference in the rate of fission in the two races must have been due to the constitution of the organisms.

We have consequently demonstrated that marked variations in the rate of fission may appear quite suddenly in the progeny of a single individual without conjugation or encystment, that some of these variations are heritable, and that they can probably be produced by subjecting the individuals to abnormally high temperature.

By referring to the table it will be seen that the mutation investigated originated, as previously stated, at the close of a period of extraordinarily high rate of fission and immediately after a short period of very high death-rate in which all but one of the lines died out. At the beginning of this period and at the close of the preceding period the individuals were very small and showed all the characteristics in behavior common to individuals about to conjugate. Whether or not anything in the nature of a nuclear reorganization in preparation for conjugation occurred in these didinia is not known, but ordinarily such phenomena do not begin until some time after union takes place in conjugating specimens. Moreover, the period between fissions was not long enough to admit of much in the way of reorganization aside from what ordinarily occurs during the process of fission. Whether or not the ancestors of the mutants were actually homozygous is not known. If they were not the mutation may possibly have been due to a rearrangement of unit characters represented in the chromosomes during fission resulting in a change in dominance. However, if this did actually take place it is not in accord with the results obtained in very extensive investigations, all of which seem to show that changes in dominance do not occur in asexual reproduction. It is probable, therefore, that the mutation was due to a direct effect of the environment on the physiological processes in the organism and not to inherited nuclear phenomena largely independent of the immediate environment.

The mutation theory so ably championed by DeVries has of late lost greatly in prestige, owing largely to the contention that the plants (*Œnothera*) in which DeVries discovered mutations were hybrids. If the conclusion reached in this work proves to be correct it will strongly support the theory in question. It will demonstrate that marked variations may appear suddenly in organisms reproducing asexually, that such variations may be heritable and that they may have a decided evolutionary value.

This conclusion, though in opposition to a great bulk of the experimental evidence gathered by some of the foremost biologists, Johannsen, Maupas, Morgan, Castle, Jennings and many others, is supported by some of the results obtained by Barber (1907), Calkins and Gregory (1913), Middleton (1915) and Jennings (1916).

SUMMARY

In a race of didinia originating from a single individual there suddenly appeared a heritable variation in the rate of fission. This variation occurred 721 generations after conjugation and 197 generations after encystment.

Two strains were isolated from this race and kept under observation for 315 days. During this time the lines in one strain produced an average of $838 \pm$ generations ($2\frac{2}{3}$ per day) and those of the other $634 \pm$ generations (2 per day).

THE METHOD OF EVOLUTION FROM THE VIEW-POINT OF A GENETICIST[1]

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A SYMPOSIUM upon the method of evolution, participated in by students from widely different provinces, can hardly be expected to develop harmony of opinion. But to be assigned a place in such a discussion as a representative of one field of endeavor does not imply that the conclusion one reaches will necessarily differ from that of his co-symposiasts; for the question of the method of evolution is a question of fact, and when the fact is discovered, it will be recognized as completely by the paleontologist as by the physiologist. And, in particular, to be assigned the final place in the argument does not mean that the doctrine preached will be, or even is, the last word on the subject. In the present state of knowledge of biology the most that can be expected of an address on this subject is a statement of principles which should guide us in a search for the facts. Beyond these principles there are justifiable suspicions, and there may even be militant conjecture, but little else.

The first fundamental principle for the guidance of one who would find the method of evolution is a principle common to all sciences which seek to explain the occurrences of a remote past. No agencies may be assumed to have operated fifty million years ago of a different order from those that operate to-day. If the phenomena of the present afford a plausible, or even possible, explanation of the past, there must be no appeal to other phenomena, the like of which do not now exist. Just as a geologist mentally constructs the rock strata a thousand feet below the surface, and the glacial drift of regions

[1] Concluding paper of a symposium on the method of evolution before the zoölogical section of the Michigan Academy of Science, March 28, 1917.

now temperate, on the basis of processes now going on in certain parts of the earth; and just as the astronomer creates the planetary systems in his mind by forces that still govern, so the biologist must conceive evolution in the past to have been the result of agencies that are still causing change to-day. Whatever causes evolution now may conceivably have caused it during the early history of living things, and there are no circumstances which compel one to devise other causes for past change.

Adherence to this principle automatically removes the first solution of the problem of the method of evolution from the realm of the investigator who deals only with past events or the results of past occurrences, and places it in the hands of him who studies present-day phenomena. Conclusions based on statistics have repeatedly shown how dangerous it is to argue from end results to causes. The compiler who finds that among the poorer classes of a population the ratio of male children to female is higher than in the well-to-do classes, and concludes that deficient nutrition causes the high male-production, might also have discovered, had his investigations borne upon that point, that the poorer classes lived in houses protected with a cheaper grade of paint or even without this protection. It would have been ridiculous to conclude that cheap paint favored boys, but that conclusion would have been as nearly *proven* by the data collected as was the more plausible conclusion involving nutrition. Causes are not safely to be judged from results. In discovering the method of evolution, the initiative is denied the paleontologist, zoogeographer and the morphologist. No doctrine of scientific cloture is here advocated, however, for the right of debate and even of veto is still theirs. The experimentalist alone may propose, but his colleagues employing the older forms of investigation may, and doubtless will, dispose. The experimentalist accepts his burden cheerfully. He knows that he may be unable to create a correct theory, but he prefers dispensing with a theory to adopting the wrong one.

If you grant that evolution in the past was caused by the same agents as cause evolution to-day, to what phenomena of living things will you apply this principle? Evolution requires two things, namely, modification and inheritance. Given these two things, the occurrence of a new characteristic and the inheritance (or even only the heritability) of the new characteristic, evolution has occurred. It matters little now what becomes of the individual or individuals possessing a new heritable character. They may even perish before they leave offspring, yet evolution has occurred. What became of these incipient races was the theme of the evolutionists of the past half century, who devised many and fanciful theories to account for their preservation or their destruction. To-day we are concerned less with the fitness of the new characteristic for the environment; we demand rather to know how the new feature arose and why it was inherited.

Fortunately there is no fundamental disagreement with regard to inheritance. Too much is known of the mechanism of inheritance to allow of dispute. The chromosomes have been saddled with the main responsibility. There has never been any general attempt to refer inheritance to the environment. No one has supposed that a goose egg laid in the sand would produce a turtle. It is true, the cytoplasm has a share in determining what shall develop from an egg; so does oxygen, and so do other components of the medium, as can be readily shown by altering those components. What develops in the presence of this cytoplasm, and out of this cytoplasm, depends specifically, however, upon the chromosomes. Disputes regarding this fact have seldom been dragged into arguments over the method of evolution.

With the primary requirement of evolution, the production of new characters, matters have been otherwise. The mode of origin of modifications has not shared the good fortune of the mechanism of heredity. There is no need to cite the hosts of opinions that have been held by reputable scientists regarding the inception of evolu-

tionary change. They have ranged from those who would make the living world of to-day wholly the product of the environment, to those that deny any participation of external factors in the course of evolution. Where in this array of opinion is the probable truth? To answer this question will be to express another opinion; but it is possible to formulate an opinion which is based upon principle, and which will therefore be more inviting than mere conjecture.

With the aid of Sir Charles Lyell, who more than any one else has taught us to seek the explanation of past events in present processes, let us look about us for the cause of diversity among individuals. We may ignore differences which, from their fundamental nature, are not permanent, that is, modifications which are not heritable; for of such as these evolution is not made. Since inheritance depends upon the continuity of material of the chromosomes of the germ cells, changes in adult structure or function can only be permanent when they follow a corresponding change in one or more chromosomes. These chromosomal changes may conceivably arise from within, or be impressed from without. Much of the modern investigation which has a bearing upon the method of evolution is concerned with the question whether the modifications of chromosomes are caused by internal or external agencies.

Two of the most striking cases of the origin of new heritable characteristics are those of the fruit fly Drosophila and the evening primrose Œnothera. Scores of permanent changes in these organisms have appeared within the last decade. These new features have appeared in one individual among a hundred in the same bottle, or among a thousand in the same field. Environmental differences seem excluded in these cases. For, if one attributes these changes to invisible and unsuspected variations of the environment in circumscribed regions in a bottle or field, there is no need to appeal, as has usually been done, to the grosser elements like climate and medium, and

evolution is once more made wholly speculative. Inasmuch as the very instability of protoplasm, which accounts for the manifold metabolic processes that characterize living things, makes not only possible but highly probable alterations of the chromosomes which, in our present state of knowledge, must be regarded as of internal origin, the discovery of cases like those of the fruit fly and the evening primrose, in which environmental agency is apparently inadmissible, should leave no doubt that evolution *can* occur without reference to specific elements of the outer world.

What the actual method of producing changes in the chromosomes may be can only be conjectured. Morgan and his students have abundantly demonstrated that the continuous identity of chromosomes is, in at least one animal, an invention based on appearances; that the chromosomes of one individual are often not identical with those of its parents. The crossing over which they postulate is an even exchange of corresponding parts of two chromosomes. How this exchange is brought about, whether through the twisting of the chromosomes as the students of Drosophila have assumed, or because of the variability of the forces that hold the chromosomes together, as Goldschmidt (1917) suggests, is immaterial. If, occasionally, this exchange between the chromosomes is not equal, an occurrence that is not inconceivable, a chromosome might be produced unlike any that ever existed. If the germ cell containing such a chromosome were capable of producing a viable individual, to predict the probable nature of such an organism would be idle speculation.

Failure of the chromosomes to divide, and the passage of one or more of them bodily to one end of the spindle, would produce daughter cells with an unequal complement of hereditary material. Hyde (1916) has recently reported a case in Drosophila which is probably of this nature; the two X chromosomes appear to have remained undivided, going to opposite daughter cells, resulting in

the production of right and left eyes of different sex-linked colors. If, among the descendants of such unusual cells, germ cells should be produced, a new type of organism should result from their development. Although Babcock and Lloyd (1917) reject somatic segregation because one supposed case of that phenomenon reported from the Oregon Agricultural Experiment Station proved, in their opinion, to be something else, there is still some evidence, like that of Hyde's, that such unequal divisions do occur. If they occur in the line of the germ cells, new modifications may thereby be produced in the next generation.

Failure of the maternal and paternal chromosomes to separate in the reduction division of maturation, a phenomenon discovered in Drosophila by Bridges (1913) and named by him non-disjunction, may also be the cause of occasional evolutionary changes.

The foregoing chromosomal irregularities, which have the appearance of being mechanical rather than chemical phenomena, are not, however, necessarily the instruments with which permanent changes of organization are wrought. Probably they are not the usual ones. They have been mentioned first because there is evidence that such changes are occurring now. Minute changes far below the present limits of visibility are as conceivable, and in my opinion quite as probable, as the grosser ones named. In what these changes occur no one knows, for no one knows the nature of the hereditary elements. Suggestions involving enzymes and side chains have been made. These are only conjectures, but they reveal a belief that the phenomena of inheritance are chemical phenomena. If we believe that heredity is dependent upon chemical processes, there seems to me no escape from the assumption that evolution is first of all a chemical change. What the cause of these changes may be is another question; but if changes in considerable fragments of chromosomes, or even in whole chromosomes, can occur as a result of agencies within the organism, as

is plainly the case in Drosophila to-day, there is no reason to deny that the invisible modifications of chromosomes, if such occur, are likewise of internal origin.

In suggesting possible sources of internal change resulting in evolution I am not blind to the fact that an ultimate explanation of the method of evolution is not thereby offered. The chemical processes which cause these phenomena, while they are distinctly within the field of the geneticist, are not within his knowledge. If there were any prospect that an ultimate solution of the problem of the causes of germinal changes could be offered at the present time, invitation to participate in this discussion should have been extended to a physiologist; for it is from him that the eventual explanation of these internal changes must come.

In this account of possible ways in which changes in the chromosomes of germ cells arise, I have not forgotten that it is conceivable that the changes are forced by external agents. There are, indeed, biologists who regularly attribute such changes to environment. The paleontologists not infrequently seem to regard evolution as ordinarily so caused. But with a few exceptions, those who hold these views are not experimentalists. They are not the biologists who are engaged in studying present phenomena. They reason from results to cause. Out of the conceivable causes they have picked on one which has a chance of being the right one, but only a chance. I venture to suggest that the theory of internal origin of modifications will account for all paleontological, morphological, and geographical phenomena, and accord with all evidence from those fields, quite as well as the environmental theory.

Among the experimentalists, it is to be admitted, there are a few who occasionally proclaim the discovery of a modification produced by the environment and subsequently inherited. By one or two, not possessed of the still small voice, these proclamations are made repeatedly. Sometimes the effect of the environment is admit-

tedly directly upon the germ cells, and the results are not usually challenged. In other cases it is claimed to be only upon the soma, which then modifies the germ cells. These latter claims, however, meet with singular indifference or even distrust on the part of other biologists. Vulnerable places are too easily found, such as the lack of adequate controls. Sometimes the environmental evolutionist is charged with unwillingness or inability to show his hand when pressed for further information. Furthermore, it may seem strange that in a world of biologists, all anxious to solve the problem of the method of evolution, and all so far as I am aware willing that that method should be anything whatever, all of the important supposed cases of permanent modification caused by environment should be advanced by a handful of investigators.

To conclude: We have affirmed our adherence to the principle that evolution in past time is to be explained by phenomena that occur to-day. No processes that do not occur in living things now may be assumed to have occurred in living things formerly, unless there is plain evidence that events not explainable in terms of modern metabolism once occurred. Applying this principle only to the origin of modifications, not to their preservation, we have shown that animals are evolving now through agencies within themselves, independent of the environment. Whether environment also produces permanent modifications is questionable, with the burden of proof still resting upon those who hold that it does. All of the known steps of evolution *may* be explained as originating from within the animals' organization. There is no necessity of appealing to any other mode of origin, except, perhaps, to satisfy a certain type of imagination. In view of these considerations, it seems not illogical to me to suspect that evolution, at least among all but the very low animals and plants, is usually if not always initiated by a chemical change, either directly or indirectly produced, in the chromosomes of the germ cells; that these

changes are inherited because they result from changes in the chromosomes, and for no other reason; that such changes are usually, if not always, independent of the environment; that such changes produce unpredictable changes in adult structure or function; and that these changes have no reference to the usefulness of the change in the evironment in which the animal exists or in any other environment.

If one desired to go beyond the first steps of evolution, and discuss the factors that determine the course of evolution by effecting the survival or destruction of such new forms, it would not be difficult to maintain that survival is much less dependent upon fitness than is commonly supposed, and that natural selection probably operates only to eliminate the most unfit. But such a proposition necessarily involves much speculation, with comparatively little information regarding present day phenomena to serve as guide. I shall content myself, therefore, with the above categorical statement of views regarding the origin of permanent modifications, and allow my colleagues to begin the sifting and testing operation which is theirs to perform.

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SHORTER ARTICLES AND DISCUSSION

AN INTRINSIC DIFFICULTY FOR THE VARIABLE FORCE HYPOTHESIS OF CROSSING OVER

THE assumption of a "variable specific force," made by Goldschmidt,[1] may seem to account for the frequency of the crossovers occurring in a given simple case of linkage; but when this explanation is extended to the results which such crossovers give when bred, it creates a difficulty of the same type and magnitude as the original problem of crossing over, for which, therefore, it is not a satisfactory solution.

Briefly put, the explanation advanced by Goldschmidt assumes that the genes are carried by the chromosomes, and that each gene is incorporated in its characteristic locus by virtue of a force residing in the chromosome and possessing properties specifically related to the properties of the genes of that locus. In the heterozygote Gg (see accompanying figure, line 1), the two forces F_G and F_g residing in the homologous chromosomes C and C′ possess not only a locus specificity but also an allelomorphic specificity corresponding to the allelomorphs G and g. When the chromosomes of the Gg heterozygote go into a resting stage, these forces F_G and F_g relax, so that the genes G and g become freed. When the chromosomes are reassembled preparatory to division these forces again come into play with the result that gene G is again incorporated into the chromosome in which F_G resides, while gene g is likewise reincorporated into the homologous chromosome characterized by the presence of F_g. In order that crossing over may occur, the allelomorphic specificities of forces F_G and F_g must, in the first place, be commensurable variables; *i. e.*, forces F_G and F_g must vary in that property which constitutes their essential difference, and in such a manner that when all the values of force F_G are represented by a characteristic frequency distribution and likewise all the values of F_g are represented by a second specific distribution, these two distributions will have a common base (see diagram, line 1). In the second place, these two distributions must overlap on the common base line so that a value chosen from the lower range of one may

[1] Dr. R. Goldschmidt, "Crossing over ohne Chiasmatypie?" *Genetics*, 2: 82–95.

be of the same magnitude as a value taken from the upper range of the allelomorphic distribution, though *the two forces thus chosen are,* of course, *no more identical than are two of Johannsen's beans which are of the same size but belong to different pure lines.* It is then assumed that in those cells in which the values of F_G and F_g are equal, the chromosome carrying F_G should incorporate gene g as frequently as gene G, and in those cells in which their normal order of magnitude is inverted, the crossover incorporation should occur more frequently, depending on the amount of inversion. Let us assume that in a given case this overlap is of such a per cent. that one per cent. of the gametes are crossovers (%1 of the diagram).

Now, in order to present the crux of the matter, let us proceed with the analysis of the behavior of the crossovers produced in the above experiment (see diagram, line 2). Let us mate a cross-

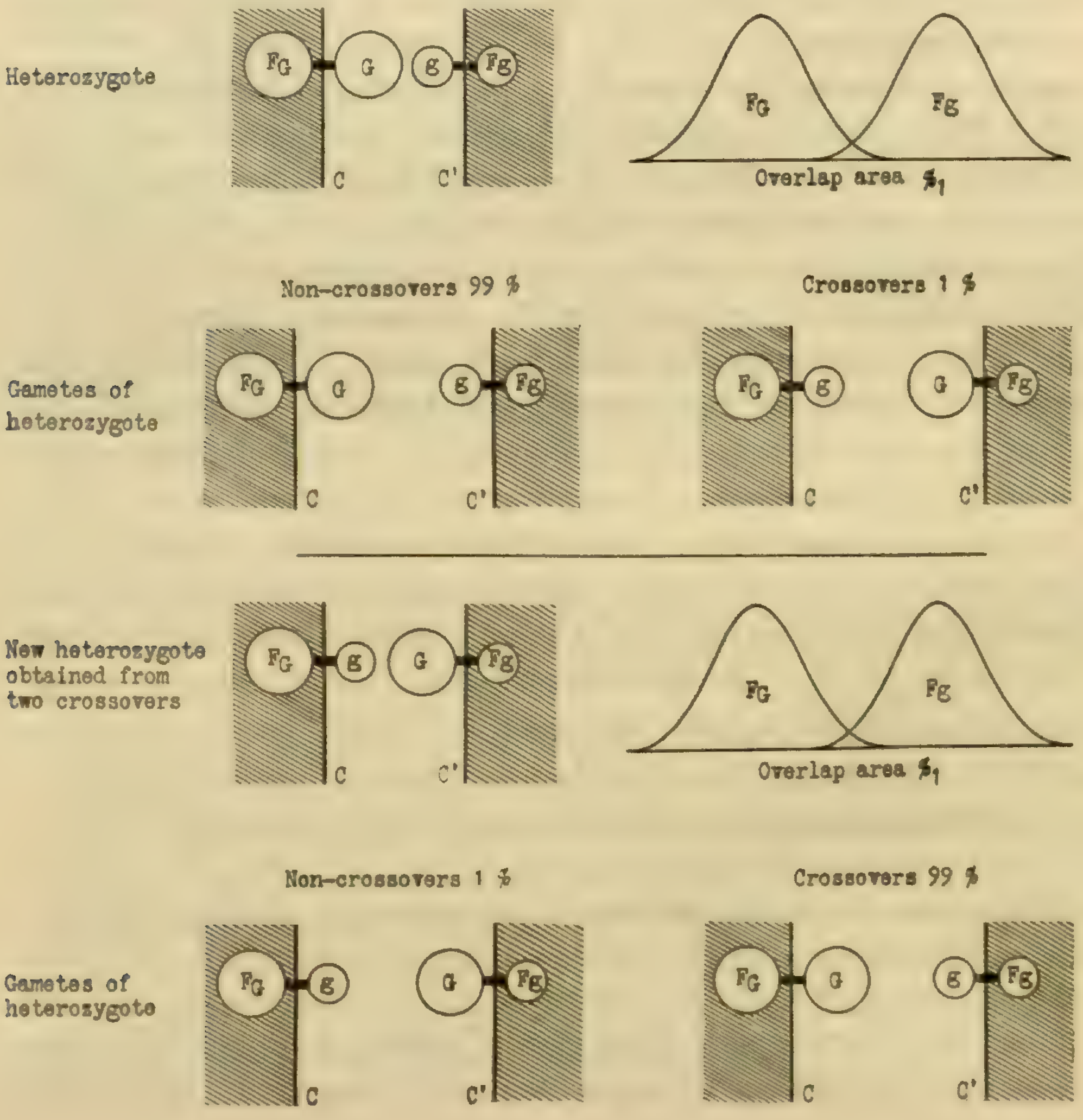

FIG. 1.

over individual in which gene G is held incorporated by force F_g with the converse crossover individual in which gene g is held incorporated by force F_G (see diagram, line 3). As soon as the chromosomes of the resulting heterozygote enter the proper resting stage the forces F_G and F_g relax, freeing the genes G and g. It must now be recalled that every value of force F_G is a member of a specific frequency distribution representing the entire behavior of F_G, and that any particular value of force F_G should give in succeeding generations the same result as every other value of F_G. Each value of F_G, whether chosen from the extreme upper range, the extreme lower range, or the mid-region, should give rise among its descendent cells to a series of variates which reproduce the original distribution F_G and no other. That is, the two distributions which describe the variates of F_G and of F_g in the cells of the new heterozygote, being specific, overlap in exactly the same fashion and to the same extent as did the distributions of the forces F_G and F_g in the original heterozygote (see diagram, line 3). Consequently, when the chromosomes are reassembled force F_G will, as before, incorporate gene G in 99 per cent. of cases and gene g in 1 per cent. of cases (see diagram, line 4). *But gene G entered the heterozygote as part of the chromosome possessing force F_g, hence the 99 per cent. of emerging offspring in which gene G is incorporated by the chromosome bearing F_G or gene g by the chromosome bearing F_g are crossovers.* As everyone acquainted with linkage knows, the crossovers given by the heterozygote from the mating of two crossovers are of the same frequency as in the original experiment. The intensities of coupling and of repulsion are *equal* and not complementary. Goldschmidt's machine which at the first revolution turned out a mere driblet of crossovers, should overwhelm the operator with a deluge of crossovers at the next turn of the crank. The whole explanation fails unless some added agency be devised to take over the duty which the specific allelomorphic forces abandon after the occurrence of crossing over.

The original problem was to secure the replacement of gene G in chromosome C by gene g, and at the same time the replacement of gene g in chromosome C′ by gene G. Having assumed the machinery of specific variable forces to accomplish this interchange, we find that the products of the interchange are not stable, and furthermore they give a result the opposite of that demanded by the well known facts of linkage. In order that

gene G should be stably related to its new position in chromosome C′ it must be held incorporated by force F_G and not by force F_g as is the case. Added on to the original problem of the interchange of the genes is now the second and equally imposing problem of the interchange of the forces subsequent to the interchange of the genes. An actual bodily interchange of the forces seems impossible in view of the assumptions we have had to make as to their nature and action. The transformations would then have to be accomplished by some transmutation *in situ*. It is evident that no internal autonomous change short of a complete and absolute mutation of force $F_G^{\backsim}$ in chromosome C into F_g and simultaneously of F_g in C′ into F_G would suffice. But we have no precedents for assuming such reciprocal mutations, and if we had, we could have sidetracked this whole machinery by applying this reciprocal transmutation idea to the genes and thereby solved the first problem in such a way that the second could not arise. Instead of localizing the cause of the reciprocal transformations of the forces in the forces themselves, one might transfer it to the genes; *i. e.*, one might endow the genes with the power of causing reciprocal transformations of the forces rather than empower the forces to transmutate of their own accord. While this form of the transmutation idea carries something of an air of plausibility, it can not be taken as more than an attempt at formal escape from the difficulty—a lifting of one's self by one's boot straps that makes more demand on credulity than, for example, one would in assuming crossing over offhand as a specific property of genes which needs, as support, only such formal explanation.

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ON THE PROBABLE ERROR OF MENDELIAN CLASS FREQUENCIES

An old friend of geneticists who dislike excessive calculation has recently been attacked by Pearl,[1] viz., the familiar formula, $\sigma = \sqrt{npq}$ for the standard deviation of a Mendelian class frequency. He proposes to substitute a more refined but much more complicated method, originated by Pearson. In a Mendelian illustration he obtains a result which differs by over 40 per cent.

[1] Pearl, R., "The Probable Error of a Mendelian Class Frequency," American Naturalist, Vol. LI, pp. 144–156, 1917.

from the usual. This seems to indicate that the old method is wholly inadequate, but further examination shows that the difference is not due so much to method as to the fact that Pearl has calculated something with a different significance from the usual probable error. A cross of Mendelian heterozygotes (Blue Andalusian fowls) gave three classes of young in the numbers 14:33:11. Expectation is 14.5:29:14.5. Pearl assumes that a first sample of 58 has given exactly expectation and then calculates the quartile deviations for each class in a second sample of 58. The results are given as 3.13 for the heterozygous classes, 3.55 for the homozygotes which indicate an excellent fit of observation to expectation. By the usual method, if a first sample of 58 had given exactly 14.5 black chicks and nothing were known of any theoretical expectation, the probable error in a second sample of 58 is measured by the probable error of differences. The probable error of either sample as given by the formula $.6745\sqrt{npq}$ is 2.22. The probable error of differences by the usual formula $.6745\sqrt{\sigma_1^2 + \sigma_2^2}$ is 3.15. This does not differ appreciably from Pearl's quartile of 3.13. Neither of these methods, however, gives what we really wish to know, the closeness of fit to Mendelian expectation. We have a theoretical expectation which is not based merely on a particular sample of 58, but which should hold with increasing accuracy the larger the first sample taken. With an infinite first sample, the formula given by Pearl reduces to the usual one, $.6745\sqrt{npq}$ giving a quartile of 2.22. This is less lenient to the discrepancy between expectation and observation than the first result, but the fit is still not bad. In a second illustration which is given, we do have two samples and no theoretical expectation suggested. The usual method of comparing samples of different sizes would be to find the standard deviation of differences on a percentage basis. The percentage standard deviation for a sample of n individuals is $\sqrt{pq/n}$, for a sample of m individuals is $\sqrt{p'q'/m}$ and for differences is $\sqrt{(pq/n) + (p'q'/m)}$. The expected standard deviation of a sample of m individuals is, however, $m\sqrt{(pq/n) + (pq/m)}$ if p and q are based merely on the first sample as in Pearl's illustration. The formula given by Pearl for the standard deviation rapidly approaches this form for large values of m and n. Following are the results given by the long method, by an approximation given by Pearl and by the usual one just cited.

	Long Method	Approximate Method	Usual Method
Median	83.53	83.95	83.71
Lower quartile	75.61	75.84	75.64
Upper quartile	91.82	92.06	91.79

The usual method gives substantially the same result as the long one and a better result than the approximate method. From the nature of experimental work, great refinement in statistical treatment is often a waste of effort, and without questioning the value of Dr. Pearl's suggestion in cases in which the greatest accuracy is warranted it appears that the simple formula is still adequate for most practical purposes.

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March 12, 1917

CHARACTERS INDICATIVE OF THE NUMBER OF SOMATIC CHROMOSOMES PRESENT IN ŒNOTHERA MUTANTS AND HYBRIDS

THE pollen grains of 28-chromosome *Œnothera Lamarckiana gigas* de Vries were long ago shown (Lutz, '09)[1] to be characteristically 3+-lobed (chiefly 4-), instead of 3-lobed, as in *O. Lamarckiana* and other diploid forms. Gates has since contributed much to our knowledge of this subject. Recently Bartlett ('15)[2], in discussing the 3+-lobed condition of the pollen of 28-chromosome *O. stenomeres* mut. *gigas*, stated that these 3+-lobed grains "are larger than the triangular grains of the type" (*O. stenomeres*). It may be added that the largest, best-appearing 3- of tetraploid forms in general, is larger than the typical, best-appearing 3- of diploid, and the largest, best-appearing 3+- of the former, larger than the typical, occasional 3+- of the latter. Smaller 3- and 3+- grains are found in the pollen of both, but they are rarely perfect-appearing, and it is doubtful whether slightly imperfect-appearing grains are capable of functioning. A careful examination of the adult characters of a form, together

[1] Notes on the first generation hybrids of *Œnothera lata* × *O. gigas*, *Science*, N. S., 29: 263–267. Gates (Pollen development in hybrids of *Œnothera lata* × *O. Lamarckiana*, *Bot. Gaz.*, XLIII, 81–115, Feb., 1907) had earlier observed 3+-lobed grains in the pollen of a triploid form.

[2] "The Mutations of *Œnothera stenomeres*," *Amer. Jour. Bot.*, 2: 100–109.

with a microscopical examination of the pollen of 10–15 buds from different parts of the plant will enable one to estimate its probable somatic chromosome number; this estimate becomes more trustworthy when one considers also (using *Lamarckiana* as the standard for comparison) the number of seeds produced per fruit by selfed flowers, the percentage of seeds which germinate, and the hereditary behavior of the plant.

I

All parts, or most parts, slender; pollen consisting of small, 3-lobed grains with an occasional 3 + -. Plant will probably be found to be diploid, or approximately so, having 14 or 15, possibly 16, chromosomes.

A. **Abundance of pollen, 50 per cent. or more of the grains good-appearing. Abundance of seed secured from selfed flowers, germinating about as for *O. Lamarckiana* when sown in soil; all offspring, with the exception of a few mutants, duplicating the characters of the parent. Plant will probably have 14 chromosomes.**

B. **(*a*) Anthers barren as for *Lamarckiana lata*, or (*b*) variable amounts of pollen produced in different buds of same plant; entirely absent in some, present in small quantities or in moderate amounts in others. In best buds, imperfect-appearing grains considerably in excess of good-appearing; in others, greatly in excess of them and in still others, very few good-appearing grains. Fewer seeds obtained from selfed flowers than from an equal number of selfed *Lamarckiana* flowers; lower percentage capable of germinating, when sown in soil, than is usual for *Lamarckiana* seeds. Plant will probably have 15, possibly 14^{+1}, or even 16, chromosomes.**

II

Plants intermediate between *O. Lamarckiana* and *O. Lamarckiana gigas* in stoutness of all parts; pollen absent, present in small quantities, or in moderate amounts, much as for I *B* (*a*) and (*b*). Pollen containing a mixture of 3- and 3 + -lobed grains, the former exceeding the latter in number. Largest grains larger than typical, best-appearing grains of diploid forms; relatively few of the grains good-appearing, even in best buds. Selfed flowers produce no seeds or very few; when seeds are sown in soil, very small percentage germinate. Plant probably triploid, or approximately so; probably 21-chromosome form if offspring of selfed 14-chromosome plant, or of 14 x 28; possibly 20 or 22 chromosomes. If produced by a selfed 15-chromosome form, or by 15 x 28, the chances of its having 22 are greatly enhanced.

III

All parts stouter than for triploid forms; pollen grains characteristically 3 + -lobed, with relatively few 3-lobed (typical grain 4-). Largest and best-appearing 3- and 3 + -lobed grains larger than typ-

ical, best-appearing 3- and occasional 3 + -lobed grains of diploid forms; 40 per cent. or fewer, good-appearing. Moderate amount of seed obtained from selfed flowers. Seeds large, germinate quickly. Plant will probably have 28 chromosomes, particularly if an offspring of a 14- or a 28-chromosome form, selfed, or of a 14 x 28. If the product of 15 x 28, it may have 28, 29, or even 30, chromosomes, I. Forms which are approximately, but not precisely, tetraploid, may be wholly male-sterile.

These statements are not intended to imply that all diploid, triploid and tetraploid forms have the characters enumerated above, but merely that forms displaying certain pollen conditions and vegetative characters will *probably* (by no means certainly) have the number of chromosomes specified.

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ON THE PERIODIC SHOREWARD MIGRATIONS OF TROPICAL NUDIBRANCHS[1]

MANY northern gastropods, including nudibranchs, are well known to exhibit the habit of congregating in shallow water along the shore at their time of breeding. This has been commonly interpreted as the result of migration from deeper water at the approach of the egg-laying season. Certain species, at any event, are from time to time found in great quantity at shore stations which they do not frequent at other periods, and field observations have apparently established beyond a doubt that this inshore appearance is closely connected with mating and oviposition. The migration into shallow water, or other means which accomplishes the shallow-water flocking in these cases, may be regarded as a device which insures the concentration of individuals within a relatively small area, thus tending to make more certain the chances of pairing in a large number of instances, as well as a method of determining favorable conditions for larval development.

Collectors of nudibranchs who have worked in tropical waters have also reported cases which at first sight seem to afford additional examples of the coincidence of the spawning period with appearance in great numbers in the littoral zone (*e. g.*, Crossland, quoted by Eliot, 1904, p. 87). While engaged in working along the shore during a period of some days or weeks, it is

[1] Contributions from the Bermuda Biological Station for Research, No. 59.

noticed that a certain species of nudibranch, until then found sparsely, if at all, suddenly begins to occur in abundance. It is also observed that at this time these nudibranchs are depositing eggs in the field, or that they pair readily and lay egg strings when kept in aquaria. The inference which has been drawn in such cases, namely, that the appearance in shoal water is in some way intimately related to the mating process, seems legitimate enough.

But I have observed at Bermuda certain facts regarding the normal migrations of a member of the typically tropical genus *Chromodoris* which, it seems to me, cast considerable doubt on the theory that this species, *C. zebra* Heilprin, moves into shallow water for the purposes of mating and egg deposition. The facts in this case, so far as they have been observed, are briefly as follows:

It was necessary to obtain considerable numbers of *C. zebra* for use in experimental work (Crozier, 1916[a], 1916[b]); consequently collections were made at short intervals (every day during some months) over the period from August, 1915, to October, 1916. I had had occasion, also, to note the occurrence of this species in the summers of 1913 and 1914. In June, and during the early part of July, *Chromodoris* was found in great abundance upon the "eel grass" in certain tidal "creeks" (Fairyland Creek, Millbrook Creek). Subsequently, in the last two weeks of July and in August, they became very scarce in such places, although a few could almost always be discovered by careful searching. At other times of the year a supply of the animals was obtained on hard, open bottoms in somewhat deeper water (1 to 2 fathoms, at low tide), in places where, I am certain, they would never have been seen during ordinary shore collecting. Occasionally, however, as was noted particularly in December, 1915, *Chromodoris* was abundant along the rocky shores of smaller islands, ranging well up to low-water level.

It seems clear enough that in *Chromodoris zebra* there undoubtedly does occur from time to time a movement of numbers of individuals toward the shore. But there are several facts which sharply contradict the view that this migration is connected with reproduction. The nudibranchs pair in the laboratory and lay strings of fertile eggs at all seasons of the year (cf. also Smallwood, 1910), and not merely at the times when

they are abundant near low-water level. Moreover, I have obtained the egg masses in dredgings at every season of the year; hence we may regard the fact of egg laying at all seasons under laboratory conditions as of significance in this connection. The eggs, which are quite characteristic in appearance, and hence easily identified, have been collected in depths of eight fathoms and more. Large individuals of *C. zebra* are likewise not uncommon at these depths; in fact, the first ones to be described were dredged from ten fathoms in Harrington Sound (Heilprin, 1889, p. 187). A further point of considerable significance is found in the fact that these nudibranchs, unlike *Elysia* and certain other species, do not appear to deposit any egg masses upon the "eel grass" on which the animals occur in such great numbers throughout the early summer. The egg strings found in the field are invariably attached to rocks, or to the shells of *Arca noe*, the "mussel" with which the adults are frequently associated. The gelatinous egg-ribbons (cf. Smallwood, 1910) are quite large, measuring usually 120 to 150 mm. long by 15 to 17 mm. broad, and are much too heavy to be supported by a blade of "eel grass," as can readily be determined by trial. It is only rarely that an egg mass has been obtained in shore collecting.

The migration of *C. zebra* into shallow water cannot, then, be directly connected with reproductive activities. Since, in the laboratory at least, they deposit eggs usually within twenty-four hours after pairing, it does not seem to me probable that these nudibranchs pair to any great extent during the time which they appear to spend in the tidal "creeks"—no eggs, as stated, having been collected from among the "eel grass," nor were any ever obtained on the muddy bottoms of these "creeks." *Chromodoris* seems to require a firm, hard substratum for the attachment of its egg-ribbon. If individuals obtained in quite shallow situations are kept singly in aquaria they sometimes deposit after several days fragments of egg-jelly containing several dozen unfertilized eggs, while they almost invariably pair readily when given the opportunity. Nevertheless, it should be stated that the nudibranchs usually do not occur singly, two or three being commonly found within a space of several square meters even when the total number of individuals in a given area is small; and I am well aware that laboratory findings with regard to breeding habits are liable to be misleading. The established

fact of egg production throughout the year in deep water is, however, good evidence that the periodic (or intermittent) abundance of this nudibranch in shoal situations can have little if any relation to oviposition.

It might at first be suspected that the periodic shoreward movement represents the phylogenetic persistence of a well-defined habit possessed by not distantly related northern species. From this standpoint, reproductive functions in *C. zebra* might be conceived of as having become dissociated from the habit of migratory periodicity, since in warmer seas, where the seasonal alteration in physical conditions is reduced to a minimum, it is well known (cf., for example, Semper, 1881, p. 135) that many forms have no specially restricted time for breeding. However attractive such a speculation may appear, it is eminently more satisfactory to regard these periodic littoral appearances of tropical nudibranchs as being controlled by definite physical influences in each individual case. Such directing causes would not necessarily be always the same for each periodic occurrence of the animals in shallow water. Although shoreward migration and egg laying are closely connected in northern forms, it is still probable that physical circumstances in the sea immediately control the migrations even in this instance also.

I have purposely refrained until now from discussing certain minor fluctuations in the littoral abundance of *Chromodoris* which are, nevertheless, important in connection with the idea that the supposed "migration" at certain times into very shallow water is, after all, only the unrestricted expression of a tendency to upward movement—negative geotropism. It has been mentioned that during the greater part of the year *Chromodoris* was collected in 1 to 2 fathoms. But after storms of some severity they were to be had only in much deeper water. The nudibranchs undoubtedly move into deep, quiet places when the surface is greatly disturbed. Just what their behavior is under these circumstances can not be stated from direct observation, for obvious reasons; and for several days, or even for a week after a severe blow, the water in the sounds and bays remains so roily that it is impossible to see the bottom. But I have frequently observed individuals creeping up from deep water after the sea has become quiet and transparent. As regards the bearing of these facts upon the major flocking into the littoral zone, which occurs in early summer, it is to be noted that the mere

continuance of quiet, still weather is not enough to determine the abundance of *Chromodoris* in the tidal "creeks," since they disappear for the most part before the calm summer season is half over. The occurrence of individuals in deep water, together with field observations of specimens which were engaged in creeping downward on the sloping sides of rocks and reefs, leads me to doubt very much that any form of geotropic irritability exerts a preponderant control over the normal behavior of these animals. My observations strongly suggest, however, that there does occur to some extent (in appropriate places) a diurnal vertical movement of *Chromodoris*, which is directly determined by the positive phototropism of these nudibranchs.[2]

Specimens of the species known as *Chromodoris roseapicta* Verrill (there is some doubt that it is really a *Chromodoris*) have been found in littoral locations, only in the summer time, but this type is not sufficiently abundant to make possible a testing out of ideas concerning its migratory movements.

The point which I wish to emphasize most is the uncertain nature of conclusions having reference to the normal behavior of animals inhabiting the warmer seas on the basis of comparisons with superficial features of the movements of their relatives in colder waters. In the case of *Chromodoris zebra*, it seems to me definitely established that the periodic flocking of individuals into very shallow water has no immediate connection with reproduction.

On Jan. 10th, 1917, I found that *C. zebra* was crowding in great numbers into the entrance of Fairyland Creek. During the next few days they became very abundant indeed, so that on one occasion 230 of them were picked up in less than an hour's collecting. On Jan. 12 I began to find egg masses attached to certain sponges, matted algæ, mangrove roots, and sundry mooring stakes in the "creek." I had not before found any in this place, as stated above. The nudibranchs were observed in copulation, and great numbers of egg-masses were found. The attachment of the egg-masses was most frequently to some firm object. Within the week Jan. 10–17 they began to disappear, and after a fairly severe storm which came at that time very few were obtainable in the "creek." This occurrence seems to form a good

[2] I am anticipating here the statement of certain facts regarding the responses of *C. zebra* which were established in this laboratory several years ago by Dr. L. B. Arey (*cf.* also Crozier, 1916[c]).

instance of shoreward movement coupled with reproductive activity, but the fact remains that the nudibranchs do breed abundantly at other times and in much deeper water.

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AGAR'S ISLAND, BERMUDA.

W. J. CROZIER

NOTES AND LITERATURE

DEAN AND EASTMAN'S BIBLIOGRAPHY OF FISHES

In order that the production and diffusion of knowledge may but be promoted, knowledge gained must be published in some permanent form. But when the publications become numerous and scattered throughout many journals, and in various languages, it becomes at length difficult, or even impossible, for any human being to retain in mind all that others have discovered and written. The literature must be organized in such a way that the seeker after knowledge and the producer of knowledge may be enabled to determine easily what has been published on any particular subject. Hence the need for bibliographies and bibliographies of bibliographies, for the *Zoological Record*, and the International Catalogue of Science. Hence the justification, the necessity, for Dean and Eastman's Bibliography of Fishes. Dean tells us in the preface that in this work there are listed more than 40,000 titles. How small a number of these could any man command were it not for some such collection!

One volume only of the work has yet appeared. This is a book of 718 octavo pages of small print; and this is occupied simply by the authors' titles of papers alphabetically arranged. And only those authors have been reached whose names begin with the letters A–K. A second volume is to follow which is to include the others. The time, the patience and the labor which the accumulation of such a list demands may be surmised by the reader of the preface; it can only be realized by one who has tried his hand at something of the kind himself.

As the work will then stand, the student of fishes can determine readily all the papers that any author, as Agassiz or Baird for example, has written; or he can glance over all the 40,000 titles and pick out those which seem to have a bearing on his subject. To obviate the latter necessity, a third volume is to follow which is to be an index to the preceding volumes. In the two volumes of authors' titles each paper is followed by the year of publication and a serial number, as "Jordan, 1891, 4"; and in the index each paper is to be referred to briefly by the author's name, the year and the serial number. Economy of labor and

expense is thus effected. The index will certainly be classified in such a way as to make it reasonably easy to arrive at the papers desired. In estimating, therefore, the work that Dr. Dean and his editor and assistants have done we must consider not only the collection and preparation of the titles, but likewise the analysis of these papers and the recording of the contents under their proper heads.

There is a need for more yet to be done. The author tells us that the index does not include detailed references to species, genera, or ever, in many cases, families of fishes. "This would entail many years' additional listing, but should unquestionably next be done." The busy student may want to know what has been written on the Centrarchidæ, or the genus Lepisosteus, or the rainbow trout; and he ought to find all of the papers recorded under each head. May the good men who have worked on this Bibliography of Fishes retain their powers and live long enough to accomplish the work.

However, it will be open to any one to go through those 40,000 books and papers and cull out the things bearing on the subject he has chosen and to publish a little bibliography of his own.

The present writer has not undertaken to discover omissions of papers or errors in quoting them. Certainly omissions and errors occur, as in any human production; but doubtless all possible care has been taken to avoid them. Two omissions have incidentally been brought to notice. The first of these is a paper by Eigenmann on a fossil species of Sebastodes, in *Zoe*, Volume I, 1890, page 17; although another paper cited ends with page 15. The other paper omitted is B. K. Emerson's "Geology of Old Hampshire County, Massachusetts," in which there is a list of the Triassic fishes found in the state mentioned. Dr. Dean must have had a record of this paper.[1] A paper by E. W. Claypole[2] is quoted from the *American Geologist*, Volume XXIX, p. 44; but the paper is not found as cited; nor elsewhere, so far as the present writer knows.

OLIVER P. HAY

[1] *Science*, Vol. XVI, 1902, p. 701.
[2] Claypole, 1890, 1.

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RATS AND EVOLUTION

A. C. HAGEDOORN, MED.ARTS, AND A. L. HAGEDOORN, PH.D.

IN treating a large group of animals from the standpoint of a systematical zoologist, it makes a very great amount of difference whether one does the work in the region inhabited by the animals, or somewhere else with the aid of collections in a museum. A real systematist, of the museum kind, does not come into touch with a number of very real problems which present themselves to field workers, and when he does, he has every inducement to brush them aside with an authoritative gesture, as he is not in a position to valuate their importance. He takes for granted that two similar skins with similar skulls which he receives from the same place, correspond to a multitude of individuals, all with these same characters; that they are a sample of a multitude of animals all exactly alike, and when he finds that animals of such a description have not hitherto been named, he can invent a well-sounding name for the two skins, and publish a description, and henceforth this description of the type specimen and this species name are welded together. If it so happens that an animal is never again collected which corresponds to the published description, the species becomes known as very rare.

There exist conventional rules, which, in the descriptions of species in certain groups, ascribe more value to certain characters than to others. In the systematic classification of rats, the points which are specially noted

in this connection are the shape of certain ridges on the skull, pads on the soles of the hind feet, the relative length of the tail, the length of molar complexes, and the length of the ears.

It is significant to observe, how every field worker who occupies himself specially with rats has his own opinion about the relative importance of these different points for the systematic classification of the animals, and discovers very soon that the work done in museums does not materially help him in his quest.

In 1915 one of us was commissioned by the government of the Netherlands to make a biological and zoological study of the rat population of the Dutch East-Indian colonies, more especially of the island of Java, with the ultimate object to find out what measures could be taken to prevent the exceptionally serious damage to public health and to agriculture caused by rats. Some preliminary work on the subject had been done by medical investigators and by a systematist working with preserved specimens in Holland. The systematic-zoological work in Java was begun some years previously by Maj. G. Ouwens, who is continuing the work after we were obliged, for reasons of personal health, to leave the tropics.

Very soon after arrival we discovered how very little the work done in European museums was to help us out in the field. We are not systematic zoologists, and our reasons for accepting the task lay in the promise the material gave of throwing light on the question of species (in which it has not disappointed us). Therefore the only group of animals with which we have at all deeply concerned ourselves with systematics is the rat, and we would not be prepared to maintain that for other groups the ordinary museum-zoology has so little value in giving a conception of the relationship between species in nature. Still, the study of rats from a semi-economical point of view has certain advantages over purely scientific collecting, as the material studied is very plentiful, and an

extraordinarily great number of keen-eyed persons, public health officials, anxious owners of coffee-plantations, managers of sugar factories, native officials in rice-growing centers, are continually observing the animals, and are more than willing to collect extraordinary large numbers on request. It is not uncommon for any one studying rats to see several hundred animals brought together for him to look over, and one of us has had the occasion to observe a batch of ten thousand rats in one day within the grounds of a sugar factory where between nine and twelve thousand rats were killed daily for several years.

The study of rats has set several authors to speculate as to the nature and the origin of species. Very prominent amongst these is Lloyd (The growth of groups in the animal kingdom). Our conclusions differ materially from those of Lloyd, however. The reason for this difference, we venture to think, lies chiefly in the fact that whereas Lloyd studied dead rats, and speculated upon the origin of his animals, more especially of aberrant types, we have been breeding rats for some six years, and have witnessed the origin of aberrant types. The examples in this paper will be found to be nearly all taken from rats.

When it is found in field work, that two species-names, each given to a skin in a museum drawer, in reality correspond to two real groups in nature, of which they are representative, we may be dealing with one of two different possibilities. It may be that the variability within the first group is not so great that individuals belonging to it fall within the limits of variability of the second group, or it may happen that two different skins in a museum belong to one highly variable group of animals, in which it is difficult to establish dividing lines. If, for instance, two skins with different names in a museum differ considerably in size, it may happen that even the largest animals of the group to which the smallest skin belongs are still very much smaller than the smallest adult individuals of the group which corresponds to the bigger skin. It may happen that two skins are consider-

ably different in a preserved state in respect to some salient character, whereas in nature this very character may be found to be so variable even within a small, closely related family of animals, that it has no value whatever for distinguishing two species. A case in point is the presence or otherwise of flattened hairs, or spines in the coat of rats. On the other hand, it may happen that two species, if once they are dried and preserved in a museum, present no, or, no appreciable, differences, whereas in reality, these two species may be found to differ very definitely biologically. As an illustration we may cite the case of the field-rat and the tree-rat in Java.

The easiest way out of the difficulty is the one taken by a great many zoologists, working through large collections of animals in museums namely, to give a new species-name to every animal which differs markedly from other described species, and which as yet goes without a name.

But if one wants to go deeper into the subject, if one wants to know whether these species of the drawer have their counterpart in as many species in the forest and field, the task becomes more difficult and even hopeless for a great many investigators. As soon as specialists take in hand some group or other, it is very soon obvious that the task of finding out just how many species they are dealing with and how they differ is very much more complicated than it looked when studying the collections in a museum, however well stocked. In treating rat material from a zoological-systematical standpoint, a number of problems confront the investigator from the very outset, and he must try to find his own solutions. Every investigator treats the material in his own way, and where one man makes fifty species, some other man will make two species out of the same material. It is evident, that if the term "species" means anything at all, it must hypothetically be possible to divide the material into a fixed number of species, neither more nor less. The vague way in which the term "species" is applied, must be chiefly

responsible for the unrestricted feeling of personal liberty which systematists undoubtedly have about the way in which they divide a number of dried animals into species. It is for this reason that it here becomes necessary first of all to give our definition of the term "species."

For numerous systematists, a "species" is the description of a skin and a skull deposited in a museum—the type-specimen—and to this species belong all the animals which have just such a skin and skull. Some few botanists are just now trying to reserve the term for a group of animals or plants which have the same genotype, the same set of inherited factors of development. As long as we concern ourselves with autogamous plants, such a definition might pass, we might, at least hypothetically, divide a population of such plants into a number of species and a few hybrid individuals.

It is very obvious that this definition of "species" falls short, as soon as we concern ourselves with animals, or with allogamous plants. In such groups, according to this definition, there would be no species. Even the genotypically purest group of animals would in every instance still be composed of two species, the males and the females, for we now know that the sex difference is caused by a difference in genotype.[1] Therefore, such a definition of the term although very concise and very short, is practically untenable.

When we say: Species are those groups of individuals, which have a common genotype, and which are pure for that genotype, we can most certainly concede to Lotsy that species are not variable,[2] but if we do so, we limit the use of the old word "species" to those groups of plants which really are pure and therefore invariable, so that they can not be changed by selection, natural or artificial.

If we solemnly state that dogs have short twisted tails,

[1] "Mendelian Inheritance of Sex," A. L. Hagedoorn, *Archiv für Entwicklungsmechanik*, 1909.

[2] J. P. Lotsy, *Handelingen van het Natuur en Geneeskundig Congres te Delft*, 1912.

we are perfectly within our rights when we use the term "dog" for bulldogs only. But such a statement brings us no insight in the shape or the length and variability of the tail in the big group of animals which everybody, excepting breeders of bulldogs, knows under the name of "dogs."

We can say: "Carriages have small wire rubber-banded wheels" and if so we are within our rights if we limit the term carriage to baby-carriages, but all such and similar statements of wheat-growers, breeders of bulldogs and manufacturers of baby-carriages, no matter how plausible they may look to the people under consideration, have this one thing in common, that they may not be generalized. Breeders of New Foundland dogs have as much right to reserve the name dog for their animals, and to say that dogs have long bushy tails, as the breeders of bulldogs did, and if we permit the manufacturers of gocarts to reserve the term "carriage" for their product, and if we allow the breeders of autogamous plants to limit the term species to species of wheat and barley and peas, manufacturers of Pullman carriages certainly have the right to state "Carriages are ninety-five feet long and are entered by steps four feet from the ground" and the breeders of sugarbeets or rye, and the zoologists will have the right to state that species are variable.

When we want to make a definition of the term "species" we must make it so that it fits rat-species as well as wheat-species, and in such a way that the geneticians as well as the systematicians can apply it to the things they are wont to call by the name.

We know that all the different genes, all the different inherited factors whose cooperation or non-cooperation to the development of the most diverse organisms produces the hereditary differences among them, are each in themselves invariable. We have called this invariability of the genes Johannsen's law.[3] Only in this way can

[3] A. L. Hagedoorn, "Wetten en Regels in Genetica en Eugenetica," *Handelingen van het Genootschap van Natuur, Genees-en Heelkunde*, 1913.

one explain that those groups of plants, which are so constituted that they become automatically pure in a short number of generation—the autogamous plants—consist in the main of pure and invariable species, which can not be changed by any amount of selection.[4] Selection within a group of plants which descends from one individual, homozygous for all its genes by a continued autofecundation, is ineffective. As we have the name "pure line" for these groups of plants, there is no good reason to limit the use of the term "species" to these groups exclusively.

Liability to change by selection is synonymous with genotypic variability, and this true variability is synonymous with impurity. Those species which do not exist exclusively of individuals which are all mutually identical in respect to all their genes, are variable and therefore liable to change by selection. One single, genotypically pure species as a rule can not give rise to new species. There have become known a few cases[5] of real spontaneous genovariation, mutation, in which every known cause for change in genotype was excluded (one of us has noted three such instances in the mouse); but as in every instance we have been concerned with a dropping out of one gene we can practically leave them out of account here. There exist pure species, but there certainly also exist variable species, species which are certainly liable to change by selection.

In evolution we are certainly concerned with two different sets of processes, on the one hand with the causes of variability, and on the other hand with the processes which limit variability.

Throughout this paper we will call *total potential variability* the quantity of genes which not all the mem-

[4] A. C. Hagedoorn and A. L. Hagedoorn, "Studies on Variation and Selection," *Zeitschr. für Induktive Abstammungs- und Vererbungslehre*, 1914. A. C. Hagedoorn and A. L. Hagedoorn, "Can Selection improve the Quality of a Pure Strain of Plants?" *Journal of the Board of Agriculture*, 1914.

[5] A. L. Hagedoorn, "The Genetic Factors in the Development of the House-mouse," *Zeitschrift für Induktive Abstammungs- und Vererbungslehre*, 1911.

bers of a group have in common, or for which they are not pure (homozygous), and the variability which this impurity makes possible in the descendants.

At least ideally, we can express the potential variability of a group of individuals in a number. There certainly exist species with a total potential variability of zero; these are, for instance, the pure lines of certain autogamous plants, those species for which Lotsy would like to reserve the term species altogether.

We will now try, by the aid of this new term, total potential variability, to give such a definition of the word "species" that it comprises everything which zoologists and botanists, geneticians and systematists, have vaguely meant by it. Our definition is as follows:

A species is a group of individuals which is so constituted genotypically and which is so situated, that it automatically tends to restrict its total potential variability.

Every group of individuals which is closed to the admixture of individuals from without, such as the descendants of an autogamous plant, the dogs or cattle in an exclusive stud, a "Paarungsgenossenschaft" of animals or plants bound by a peculiar habitat, has the tendency to become purer and purer automatically, and to reduce its variability continually. Species originate, given a certain variability of a group of individuals, through all those agencies separately or in combination which bring a group of individuals (not necessarily a small group) into such conditions that the new group has a tendency to become pure for its own genotype. We can not say in general that species are produced by inbreeding, or by isolation, or by a change of habitat, or by colonization, or by selection exclusively. An individual or a group must have a certain amount of potential variability to be able to produce a species, different from the one to which it belongs.

We know now that the genes themselves are invariable. There remain only very few authors who still believe in the variability of the genes. It is therefore necessary to

find out the causes for genovariability. Real mutation, as far as we know, exclusively consists of an occasional loss of a gene without visible cause. Mutation therefore can at the utmost heighten the potential variability by one. De Vries's conception of periods of mutation is at present only of historical interest.

In our opinion, crossing, recombination of genes by mating of individuals of unequal genotype, is to be regarded as the only real cause of variability. There is no good reason to change the opinion of one of us, namely, that there exist three different kinds of variability.[6]

A. Modification, the non-inheritable effect of the non-genetic developmental factors.

B. Real inheritable variation caused by mutation, loss of genes.

C. Real inheritable variation by recombination of genes.

Lotsy has subscribed to our statement (*loc. cit.*) with the exception that he denies the existence of loss-mutations.

We can no more say that species originate by crossing, than that they originate by isolation. New pure lines of autogamous plants, the kind of species for which Lotsy wants to reserve the term, can of course originate in the descendance of one hybrid plant. There is no fundamental difference between evolution in these plants in a state of cultivation and what it must be in nature. But in allogamous organisms, we will only in exceptional cases meet in nature the same course of evolution as in our cages or experimental plots.

Even if crossing in the widest sense is the sole cause of variabilty, we must not suppose that, as a rule, new species come into being in the F_2 or F_3 generation from a cross. If we make a hybrid between species, this hybrid individual will have a total potential variability which is at least as great as the number of genes which were *not* common property of both the forms crossed. If we com-

[6] A. L. Hagedoorn, "Autokatalitical Substances the Determinants for the Inheritable Characters," Roux' serie Vorträge und Aufsätze über Entwicklungsmechanik, Leipzig, 1911.

pose a group of nothing but such hybrid individuals we will get an enormous amount of variability in succeeding generations, and when the group gradually becomes more and more pure for an own genotype this may be a completely new one. A species may have been produced with totally new characters, possibly intermediary between the parent species in some of them. The chance that hybrids of allogamous organisms, even if they are viable and perfectly fertile, will *inter se* produce a new species is exceedingly small in nature. It is much more probable that the process of species formation after crossing is as follows:

There exists a species A, with a restricted potential variability, a set of habits and mode of living all of its own, adapted to a certain environment. As a general rule, individuals of this species A mate exclusively with members of their own species. Once in a while, small groups may split themselves off from the multitude by colonization, and each of these groups will have its own potential variability, and each will gradually become pure for its own genotype, and will be less variable than the multitude.

In the same country there exists a species B, with a slightly different genotype, a different potential variability. Species B is somewhat differently built, somewhat differently coated, compared with A, and therefore fits into a somewhat different environment. As a rule, individuals of the two species do not come into touch. Let us take as examples the grey-bellied *Mus alexandrinum* which lives in houses and on roofs in northern Africa, and the white-bellied *Mus tectorum*, which lives in trees in the same countries. The same holds true for the house-rat and the field-rat in Java, likewise for the house-rat and the tree-rat.

Even if matings between the two species furnish hybrids which are completely fertile, even in localities where two species overlap and are plentiful, the occasional hybrids will be far in the minority compared to individuals pro-

duced by matings between house-rats and house-rats or tree-rats *inter se*. If the occasional hybrids grow up, they will either become house-rats or tree-rats, biologically speaking. In the first case they will mate with individuals of the house-rat population, in the other case with tree-rats. A new group, so situated that its potential variability is bound to be reduced to produce a genotype of its own or a new species, these, a few hybrid rats will certainly not produce. A single mating of a house-rat female with a tree-rat male may be the cause for a heightening of the potential variability of the house-rat population into which the hybrids merge. Eventually this higher potential variability will be reduced again. And reversely, an occasional mating of tree-rat females with house-rat males may be the cause for a greater potential variability of the group of tree-rats to which the females belong.

If it so happens that a few animals colonize out of such a population at the time when the potential variability is still higher than ordinarily, such a colony, which will have a potential variability smaller than that of the multitude, will have a chance of having a range of variability differing from that of the multitude. Such a group may become pure in respect to a somewhat longer tail, a somewhat darker belly or a somewhat greater size, as compared to the population from which it ultimately was derived.

Very good examples of such a process can easily be found by observing the evolution of certain species of dogs or poultry under domestication. For instance, the species Airedale terrier has become variable, and therefore liable to the influence of selection in different directions, because of the fact that hybrids with Dobermann pincher in Germany, and with the Gordon setter in England, have been taken up into the species, the stud not having been closed rigorously, such as the Sloughi stud, or the Jersey cattle stud. But it must not be thought that a new, improved species of Airedale terrier has been

bred from such hybrids *inter se*. The potential variability of the collie was very small, a few years ago. Hybrids with the Russian wolfdog have been taken up into the species. For this reason the variability has been very extensive during a number of years. And at present this variability is reduced again, by selection. In the meantime the species has been changed as a whole, the fashion having changed and having made much of the possibilities afforded by the cross. The collie, which formerly was an intelligent, affectionate dog, with a head shaped like a fox; inclined to be noisy, and to run after everything; with long straight, outstanding hair, and with color ranging between black and tan, and sable, with a variable amount of white, has changed completely. The show collie now is rather a treacherous and surly dog, with a head shaped like that of a llama, silent and lazy, with hair which inclines to be soft and wavy. The color is much more variable and now includes white, slaty, creamy, and generally fade tints.

In chickens, crossing is a common way of "improving" a species, and in all those instances we happen to know, the hybrids were made by using an individual presenting a character which it was thought desirable to fix into the breed, or a certain degree of development of a character, not reached by even the best individuals. Such an individual used for crossing is sometimes a pure-bred animal of another species, but much more often a mongrel of unknown extraction, happening to be somewhat like the breed to be improved, with the exception noted above. It is the practice to breed the hybrids obtained from the cross back to the species, and their offspring again, always selecting those individuals which come nearest to the general conformation of the species, but which have the character to be fixed into the breed. For instance one may want to breed blue Wyandottes. The breeder will then take any blue fowl which happens to look somewhat like a Wyandotte, mate it into a strain of first-class white or black Wyandottes, breed the hybrids back into

his species, selecting from among their blue offspring those which are the most like a good exhibition Wyandotte, and so on, for a number of generations. It is easy to see that in such a case the general potential variability of the whole group is very much increased. It diminishes automatically again, because of the fact that in every generation a few animals produce a great number of offspring. If ten young cockerells of a new species of fowl were habitually derived from ten fathers, the progress in the direction of purification, "fixing" the breed, would be almost nothing. But as ten young males habitually have only one or on the average less than one father, in other words, as only a very small percentage of males in every generation is used for breeding purposes, automatic purification, automatic diminishing of the total potential variability of the group, is very rapid. It is to be noted that in the absence of selection, the group may become pure for almost any conceivable genotype given in the potential variability, the genotypic diversity of the first animals. Therefore any character which has received no or small attention from the breeder may turn out to be different from what it was in the species to be altered into a new breed. It is for this reason, very common to observe that a number of apparently closely related species in the common fowl, or in domestic pigeons, differ, not only in the points which are obvious to every observer, but in other minor points as well, points which need not be in any way correlative to the obvious differences. A few examples. The different species of Leghorn resemble each other very closely, differing to a casual observer in color only. But the comb of black Leghorns is noticeably larger than that in white and brown Leghorns and the ears of the black species are larger than in the brown and the white. The white Leghorn has a lesser tendency to become broody than the buff. The hens lay more eggs than those of the buff or the black breed. The plumage is generally looser and longer in buff Leghorns than in blacks.

In the Wyandotte group of species, the texture of the comb is very different in the blue kind from what it is in the silver Wyandotte. The length of the tail feathers differs very much in the different breeds. The white Wyandotte lays dark brown eggs, the silver Wyandotte lays salmon-colored eggs with minute white spots, the black Wyandotte lays white eggs.

It is very rare for new species in chickens and pigeons to come up to the quality of old established ones, unless the fashion or standard happens to change. The shape and carriage of the tail, and the general shape of the body are very much better in white Fantail pigeons than in the newer black-tailed whites or white-tailed blacks. And the shape of the new blue Wyandotte is not yet what it is in the white and the silver.

We know of only a few instances of new dog or poultry species being bred from hybrids *inter se*. In those cases the breeders had no very definite object in view to start with. This mode of origin of species under domestication is certainly not the common one. Species of cultivated animals are commonly being changed by a very noticeable conscious selection. The variability necessary for improvement is continually kept up, sometimes by deliberate, but mostly by a kind of unintentional crossing, that is to say by admitting exceptionally fine offspring produced by matings of hybrids back to the species, into the registry. On the other hand, automatic purification, automatic reduction of the heightened potential variability, is the necessary outcome of the fact that only very few and very exceptionally "good" males are used as breeders.

Species of tame animals, especially fertile ones as chickens, are easily kept apart so that excessive splitting up into secondary species is possible and even the rule. For instance, in those species in which a certain much sought quality is influenced by the internal secretion of the sex-glands, it is obviously impossible to make a pure strain in which both males and females come up to one

standard. In silver Wyandottes, the standard calls for white feathers, which are bordered by a line of black. Now the males are very much lighter than the females, so that in a pure species, in which the males are correctly marked, the hens are too dark and in a strain in which the hens are good, the males are too light. The only way out of the difficulty has been the establishment of two different species, one which produces correct males and the other which produces exhibition hens. This splitting up of a species into two is very common in chickens. Such pairs of two species are kept as far apart by careful breeders as Wyandottes and Leghorns.

In the natural state, two species, even when hybrids between them are perfectly fertile, and when the individuals exhibit no preference for mating with their kind, may keep apart, if only each group is specially adapted to an own environment, so that the bulk of the animals of each species has no chance of mating with anything but their kind.

Even if there is no adaptation to an environment to keep the multitude of the individuals of a species in their place, two species may keep apart when only the animal's habits keep them from wandering very far. In such a case the borderline, where outposts of both species mix, will present a highly variable population of hybrids of all grades, the variation becoming less and less the more we look for the animals in the exclusive territory of each species. A case in point is, we think, the case of the two woodpeckers cited by William Bateson.[7] When the species differ in only one salient characteristic, the difference between them being in the main due to the presence or absence of only one gene, intermediates must be absent. In such a case the two species may be present in more or less extensive patches, separated from each other by narrow strips of territory having a mixed population. Such seems to be the case of the black and the hooded crows in Europe. Here adaptation plays no rôle apparently.

[7] William Bateson, "Problems of Genetics," Cambridge.

In one territory, two species can coexist only if for some reason matings between individuals of different species are impossible or at least less common than matings between members of the same species, or when the hybrids are sterile.

No matter where we find rats of the Rattus group there are never more than one kind of tree-rat of this group, one house-rat, and one field-rat simultaneously present in one locality, the tree-rat living and foraging in trees, and being exceptionally aggressive, the house-rat living in houses, fearing water, and not afraid of man, the field-rat living even far from cover, scarcely able to climb and too timid to enter inhabited houses. In some regions miniature rats, belonging to the same group, but too small to mate with the bigger species as a rule, inhabit houses and fields, it being very probable that these belong to two species, a small field-rat and a small house-rat.

Such a set of three rat species, a tree-rat, a house-rat and a field-rat, we not only find in Java, but also on Sumatra, the Malay peninsula, British-India and Egypt.

It is our experience that rats of the Rattus group cross with the utmost facility, and produce fertile hybrids. We have come to the conclusion that the majority of house rats remain pure for their own characteristics, even for those which have no value whatever for the adaptation of the species to its surroundings, not because no hybrids are produced with tree-rats or with field-rats, or not because such hybrids when produced are sterile, but for the simple reason that such hybrids are so far in the minority that they disappear into the multitude of house-rats, and that the heightening of the potential variability of the house-rat multitude by such occasional crosses is only local and very transitory. The same is true for the field-rat multitude and the tree-rat population. Crossing produces for each of the three species a heightening of the potential variability, and therefore it is possible that more or less temporarily, there come into being small colonies of somewhat aberrant house-rats or field-rats, in isolated places.

In Solo and Djocjacarta in Java, the great tobacco-growing companies erect enormously big sheds constructed of a very complicated scaffolding of heavy bamboo, with a thick thatched roof made of palm leaves. Such sheds are erected in the midst of the fields, mostly far from native villages. The native laborers leave food about the structure, so that it very soon becomes inhabited by rats. Now the rat population of these drying sheds is always composed of house-rats; field-rats are too timid to live permanently in places where human beings move about so much. But as the sheds are built in isolated places, they do not get their house-rat population as such from neighboring houses. We are convinced that into the composition of such rat populations, field-rats, and hybrids between field-rats and house-rats enter to some extent. This is the explanation of the fact, that very often the rat population of such a shed is found to be composed of an aberrant type of rats. If the population of such a shed becomes very numerous and a native village of some sort springs up in the immediate neighborhood, the aberrant new type may have a swamping influence upon a minority of typical house-rats brought along by the natives, so that the type may become locally common, and temporarily supersede the ordinary house-rat.

We remember Major Ouwens showing us great numbers of white-bellied house-rats, received from a tobacco-growing firm in one of the big centers, Klaten.

When there exists in a certain locality an abundant population of rats of a certain species, immigration of a few rats belonging to the same group but to a different species will have no effect. And of course it will make no difference whether the multitude belongs to the common species and the few immigrants to an aberrant new type, or reversely, as in the case of the rats in the tobacco-sheds.

Ships may occasionally bring rats to Java, from English India, or from Australia or Singapore, but the rat fauna of Java will not be enriched by a new species, as

the result of such an importation. At the utmost, the result will be, that the rat population of the warehouses in the port of entry will become somewhat more variable. It may happen that a single warehouse, empty of goods and rats, will receive a small colony of imported rats with a load of rice and rattan, but on the type of the rats of Java such an occurrence will be of no importance.

A very different thing must happen, when rats from ships come ashore in places where there is as yet no population of rats of that same group, for instance, on newly settled islands. There the imported rat population will gradually become constant, but as often for an own, new set of characters, as for those of one of the species, which originally went into the composition of the ship's population. The rat population of the bigger ships very often is a very peculiar one. It is not uncommon to find a very homogeneous lot of rats on board a ship, for which no corresponding type specimen can be found in any museum. In other instances the population of a ship may be very heterogeneous indeed. The rat population of a ship originates from rats which come aboard with cargo in the most diverse places. By crossing of such animals, all kinds of new types may arise. The rats on board a ship live under very peculiar circumstances. As the animals can not emigrate, their number is absolutely dependent upon the kind of goods stowed in the ship. For a time the circumstances may be so favorable for a multiplication of the animals, that the ship is speedily overrun with rats. Especially is this the case when part of the load affords good hiding places, such as rattan bundles, and if food is abundant, as in a load of copra. On unloading part of the cargo, a famine may result, from the effect of which all the animals, excepting only a very few, may succumb.

The result of such a catastrophe, especially of a series of catastrophes, alternating with periods of plenty, must be, that the population, no matter how variable at one time, must very quickly become pure for a genotype of its own. The occurrence of rats on board of ships is so

common that it must be an exception when a ship transports a number of rats from one port to the other without changing the type.

The rat population of a frequented port can not be taken as typical for the country where the port happens to be situated. It is always easy to find new types of rats for museum collections in cities having much shipping. But it goes without saying, that such animals, with aberrant coat characters, aberrant tail length, aberrant type of skull, perhaps, may not be called species without further ado. It is very possible that at the present moment there exist in Sourabaya twelve still undescribed types of rats, which exist nowhere else on Java. But it is probable that after ten years, thirty-five generations, those types will have all made place for an additional dozen of completely different aberrant types.

Such new types have on Java, which is thickly infested by rats, no chance as house-rats, no chance as field-rats, no chance as tree-rats. A little better is the chance which species have, whose habits of life adapt them to a special environment, where they have little or no competition to fear, or at least only from species which have such a genotype, that they do not mate with the invading species.

Mus norvegicus does not mate with animals of the Rattus group and therefore this species can, without being annihilated, penetrate into a region which is already settled by Rattus rats. We have tried to product hybrids between *Mus rattus* and *Mus norvegicus*. We put eight males and ten females of *Mus rattus* into a large cage, and when we observed the animals mating, we took out the females, and substituted an equal number of *Mus norvegicus* females. The males kept on copulating, but although we saw numerous apparently normal matings taking place, we never got a pregnant female. There is, as we could observe, no real antagonism between *Mus rattus* and *Mus norvegicus*. It is our experience that if we put two Norvegicus rats who do not know each other in a small cage together, there seldom is any serious fight-

ing. As a rule nothing happens in particular when we introduce a Norvegicus to a Rattus rat. But if we put in one cage two Rattus rats which are strangers to each other, they almost always start a fight, and generally it is a matter of life and death.

Mus norvegicus is a real water-rat, sewer-rat, field-rat, and in some parts of Java, where the poor houses have no floor, and where there are many covered sewers, as in Solo and Sourabaya, it becomes a house-rat in a certain sense. But it takes extreme negligence of the inhabitants of a house to make it shelter this rat. It happens that in houses where the bedstead is never moved from its place, and where the space below it is used as a place to dump the garbage, that this rat establishes itself there, excavating numerous large burrows.

It is very remarkable that this rat, which is extremely uniform all through its range in Europe, is very rare in this island, where the geographical distribution shows it to be a recent immigrant, which has come in by way of the big rivers. The skull, the shape of the parietal ridges, the relative size of the bullæ, the relative size of the molars, the relative position of the choane, characters which are very constant in Europe, become very variable here. In size it varies very much, the biggest individuals weighing nearly twice as much as the biggest European animals. The color, which hardly varies in Europe, varies between very light gray agouti to a silvery blackish dark gray, with dark belly there. It looks to us very probable that this great variability may be the result of crossbreeding between this species and species of Gunomys or Bandicota. The variation of the species in Java is certainly towards the characters of these rats, which have a somewhat similar mode of life as *Mus norvegicus*.

As yet it has not been possible to make Bandicoots breed in cages, although we have tried to make them do so in very quiet concrete rooms. Whenever it will be possible to breed these rats it will be very interesting to observe whether *Mus decumanus* will mate with Bandi-

coots, and whether the hybrids are fertile. It seems certainly significant that almost nowhere is the "wirok" population composed of Norvegicus animals as well as Bandicoots. From one locality the people will report and send in gray "wiroks" (Norvegicus), from other localities they will send long-haired black "wiroks" (Bandicoots).

From the standpoint of a systematist, it may look as if it were hardly more than a question of education whether a man is going to follow Hossack and bring all the animals of the Rattus group to one single, variable species, *Mus rattus*, and will look upon the differences between the three main species of this group as uninteresting variations, because he finds all kinds of intermediates in a museum, or whether he is to take the opposite view with certain English museum people, and give a new species name to every couple or trio of rats of a not hitherto described species.

When we start with a drawerful of dried skins, it certainly is a matter of personal taste whether we will distribute the skins over three or ten or twenty smaller drawers, each representing a species. Systematists may quarrel about it, whether a difference in contour of a line on a skull, or a different number of scales on the tail is or is not sufficiently important to make a group deserve a species name, or whether to call it a variety of some other species.

As soon as we have to deal practically with a group of animals like the rats of Java, and have to consider the economic importance of tree-rats to plantations, of house-rats in connection with infections, and of field-rats as regards crops, the museum kind of systematics very often proves insufficient, and we have to begin the work anew in another way.

I remember that one day, among a batch of some ten or eleven thousand rats caught on that day in a sugar plantation, Ketangoengan, there were two with markedly ruddy hue, two with very long tails, three house-rats

(brought by the same boy), one tree-rat and several thousand field-rats. If we suppose a man to prepare a batch of these rats to send them to a zoological museum, this museum would most certainly receive two reddish field-rats, two long-tailed ones, three house-rats, one tree-rat and three normal field-rats. It stands to reason that these dead rats would become five species in the museum, and to anybody looking through the drawer later on, these five species must look equivalent.

By observing all kinds of rats with new characters in the descendance of hybrids, we have become very skeptical indeed in accepting as real existing species those rat species which are represented by two or three skins in a museum, such as, for instance, *Mus Blanfordii,* or *Mus Diardii.*

It is possible that two real species, in the sense that they are real constant types, which remain constant and return to constancy after a cross which heightens their potential variability, not infrequently intercross, the hybrids always disappearing again into the multitude of typical individuals of either species.

The finding of such hybrids has undoubtedly confused the species question very much; on the one hand, several hybrids or sets of hybrids of the first generation, as well as "back crosses" must have been described as species, whereas, on the other hand, some naturalists, through the observation of such intermediate individuals, linking the types of the parental species must have come to the conclusion that they were dealing with only one varying species.

We must never forget that, though certain systematists may think that they can divide a chest of skins, according to their taste or even after profound morphological or biometrical studies, into two or six or sixty species, in reality the boundaries between species in nature are far from arbitrary. And species are really existing genuine groups, with natural, permanent limits.

There do exist very peculiar groups of animals, poly-

morphic species. Whereas polymorphy in autogamous plants really means the existence of a multitude of pure lines, a great many closely related pure species of plants which can easily be seen to originate through occasional crossbreeding, polymorphic species in animals and wild-flowering plants seems to be fundamentally different, in that there is a continual crossbreeding going on without the corresponding automatic purification which we see everywhere. Such species as the ruff and some grouses are always as variable as ever. The street-dog population or the population of non-selected cats in any large town will furnish examples of polymorphic animal species.

Now one of the chief factors in the diminishing of the total potential variability of a group is certainly the fact that a given number of animals in one population are certainly not the descendants of a number of parents of the same magnitude, but of a very much smaller number of parents.

And it is easily conceived how the fact that every female mates with several different males at each conception changes this disparity. This would partly account for the continued existence of polymorphy in the ruff and in the cats and dogs whose breeding is unrestricted, and in the sugar beet.

As to the reality and the limitations, and differences of species, the only way to reach a satisfactory conclusion is breeding them. And the possibility of breeding rats under scientific control is one of the reasons why so much of the experimental probing into the species question is connected with rats.

The Javanese house-rat has a uniformly dark belly, dark feet and a long tail, and a certain ridge on the parietalia which no other Javanese rat possesses. Once in a while a rat is caught in a house or a loembong (rice storehouse) with a short tail, or a somewhat yellow tinge, or with markings on the hind feet, or with a white belly. By a study of these individuals only, it is impossible to find

out whether the species house-rat is really so variable, or whether we are dealing with a new species, or whether they are hybrids having a field-rat or tree-rat father, or descendants, backcrosses from such hybrids. The only way to get light on these questions, which are not only of interest for economics, but for genetics as well, is the making of hybrids. It is very probable that in reality there does not exist anything which corresponds to the dozens of rat species which can be found in all the museum catalogues.

Zoologists and botanists often make short work of the hybrid question, by simply calling all intermediary individuals hybrids. In reality hybrids are very often intermediary, especially when the parent species differ in a great many genes. But very often hybrids show totally new characters which would make them species in the eye of several systematicians.

We mated the small brown agouti house-rat of Java with a large yellow, rather long-haired male, descending from a complicated cross combining *Mus rattus, Mus alexandrinus* and *Mus tectorum*. The hybrids are dark grey, with white belly and orange-ruddy sides, and very much smaller even than house-rats of the same age. Rats like these from a warehouse or from a ship, especially a litter of similar ones as in our case, would certainly have obtained a new species name in a museum. It is not impossible that similar animals with a similar origin are already present in a museum under a new name, as representing a rare species. As long as we had no proof that a new alleged species of rats were not fairly constant under cultivation, and produced a not too variable descendance, we would not accept it as a good species. And even so, we would require to know whether there were anything in its habits of life, or in its relation to other species, which warranted a belief that it would not be swamped in a few generations. For the only thing which distinguishes a species from a variety is the automatic permanency of species as compared to the relative inse-

cure standing of varieties. If all the dwarf mice in a given haystack have white tail tips, because of the fact that the first two mice which happened to find the stack when it was newly made had white tail tips, we can not say that we are dealing with a new species. We have a white-tailed variety of the local species. But if we take a dozen of these mice into our house and succeed in breeding them in cages, we may say that now we have founded a domestic species. This species will continue to exist as long as men will keep dwarf mice in captivity (witness the so-called Irish rats) and long after the stack is broken up, and the few remaining white-tailed mice have been taken up into the normal species. The difference between species and varieties is not determined by the magnitude of the departure from a given type, and it is not a genetical difference. It is a difference in expected permanency. Varieties can become species by migrating into new surroundings, or by a change in surroundings.

It seems more than probable that a great many species in museums are nothing but aberrant types which fall outside the normal variability of an existing species, and have originated by crossing, one or more generations removed.

As we have already said, the only way to find out whether individuals intermediate between existing species have to be looked upon as hybrids, or descendants from hybrids, or as variants of one of the species, is by producing the hybrids and comparing them to the collected material.

It is rather difficult to get rats of the Rattus group to breed in captivity. As we did not succeed in the beginning, we rented a small vaulted room in the ruins of a castle in France, fitted it out with numerous old boxes and baskets, faggots and straw, and turned two females loose in it with one male. There we gave them enough food to last them for a week so as to disturb them as little as possible.

Later on, in Bussum, Holland, we succeeded in breed-

ing the rats in cubical houses of four feet in each direction, made of asbestos slates, and filled with rubbish for the animals to hide in. In the beginning very many animals refused to breed even in these cages, and as the animals were crossbred from the very start, we believe that a sort of very rigorous natural selection must have been the reason for the fact that after a few generations, every couple chosen could be relied upon to breed in asbestos cages, four feet deep and sixteen inches high and wide. These cages were covered with small mesh netting only on one half of the front, and they opened upon a sort of corridor which was nearly completely dark, and could be darkened entirely. In Java some of our rats even bred in small tin cages of the size of kerosene tins. In Buitenzorg the Department of Agriculture has constructed a rat-house from plans furnished by us, composed of a series of concrete rooms, so made, that the animals can be observed from a darkened corridor without knowing it, and a series of masonry tanks with wire covers. This house is used for a biological study of rats, and for experiments in cross-breeding, to determine the status of doubtful material.

It is not necessary to clean the cages very often, if only they are well filled with dry straw and not overpopulated. Disturbing the animals keeps them from breeding freely. It happened that rats of this group bred in open wire-netting cages, but in these cages the danger exists that the mother can not make the nest sufficiently dark and secluded to prevent disturbance by the male. It is our experience, that a young female who has once neglected or destroyed her litter, is almost certainly lost for further work.

As a rule the females do not leave the nest for the first two or three days, or as long as the young are crying. Afterwards, they cover the young in the evening, bury the nest under earth, if they have it, to dig it up again at the end of the night. When the young are three days old, the mother permits young from an earlier litter

to return to the nest, but exclusively the females. Only when the young open the eyes at fourteen to sixteen days, the father is permitted to return into the nest. The young males are kept out of the nest until the young are weaned.

To observe the habits of rats of this group, an endless patience is required, as the animals, which are extremely sensitive to hardly noticeable sounds and movements, habitually are active only at night. If it is possible to darken the room completely, it is possible to observe the animals in the daytime by the light of a small lantern, after rousing them. Weak artificial light seems to make hardly any impression upon rats or mice.

We have seen wild rats, mating and foraging, to continue eating or playing, even when a small lamp was waved to and fro under their very noses, whereas the same animals would be disturbed by the falling of the head of a match. A good plan is to feed caged rats only once a day, at a set hour, to which they accustom themselves very rapidly, as in this way they can be counted upon to be up and doing at a time when it is most convenient to observe them. Even wild-living rats and mice accustom themselves to a fixed hour of feeding. A drawback of the system is that when the supply of food is not more than abundant, delay in feeding of only two hours may cause the death of recently weaned, sometimes even of half-grown rats. The discouragement may be looked upon as being partly the reason of this, for these rats are extremely nervous animals. A shock, a sudden fright, may cause them to lose consciousness for a long time, and fright will often kill them outright.

To be able to observe rats of this group at our ease we tried to tame some of them. Young *Mus decumanus* taken at the time of weaning become tame, or rather stay tame without more trouble. It is impossible to get them tame by taking them at an age of six weeks to two months, when they are wild and apt to bite. Full-grown animals are easier to tame, even if wild caught.

To make *Mus rattus* tame, it is necessary to handle the

nest young before they are a week old, which is possible only if the mother is tame enough to tolerate the disturbance. In the first generations of our work we frequently used tame *Mus norvegicus* females as foster mothers. It is especially necessary to handle the young from the very start at night, when they are very much more active. Infinite patience is required for taming these rats, for if once a young rat jumps from the hand, which easily happens, as they are very nervous, it is impossible afterwards to induce it to remain on the hand. It is possible to get the bigger species absolutely tame, so that they will jump upon the owner's hand when the cage is opened, that they will come to the hand if it is held out, will feed unconcernedly, will let themselves be taken and restrained without resenting it, and that they will not let themselves be disturbed by onlookers even in mating. It is curious to note that they do not seem to know their trainer. A tame rat is tame in respect to all humans. It seems as if taming a rat takes away a good deal of its nervousness, as tame rats are very much quieter even if among themselves, and will breed in smaller cages, and grow fatter than wild ones.

Although we have had a good deal of experience in taming nervous small animals we have never yet succeeded in taming the small house-rat, *Mus concolor*. Even small blind nest young are so nervous that they can not be induced to sit still in the hand without being held. All we could do was to accustom the animals to being restrained without resenting it.

There is a very great difference in the disposition of different species of rats, even in one closely related group such as the Rattus group. The field-rats, Javanese as well as Egyptian, and from Sumatra, behave like *Mus norvegicus*, they are reckless, timid and impulsive. An escaped field-rat can be caught in a moment, because it can be calculated beforehand where the animal will run, namely, along the walls, and thus it can be driven without any trouble into a cage or catching net.

The house-rat, European as well as Javanese, is daring, calculating and relatively little nervous, and on being persecuted, looks out very well for possible hiding places, in which the animal will remain immobile for hours. An escaped house-rat is very often found with the utmost difficulty. Their disposition makes them relatively easy to tame.

The tree-rats, Egyptian as well as Javanese, try to escape from a persecutor by climbing. They are exceptionally aggressive, we have certainly been bitten more times by tree-rats than by all our other rats combined.

In our breeding experiments we used for all rats a card catalogue. Every animal has its own card, on which are noted its number, the numbers of its parents, eventual outline drawings of its markings, and the numbers of the animals it has been mated with, together with the numbers of its young born from these matings. In moving a rat from one cage to the other its duplicate card was moved with it to a receptacle attached to the cage. On the card on file the cage number is noted in pencil. Animals which are dead get a distinctive mark, or their cards are moved to the back of the file. With such cards it is very easy to find out the ascendants and the descendants of every given animal, and it is easy to arrange the card on a big table in the form of a pedigree.

We started our experiments with Rattus rats, by taking over some animals from Dr. Lewis Bonhote. From our experiments, which we are about to describe, it became clear, that crossing is not only the means of recombining the characters of the species crossed, as many English authors have it, but that absolutely new characters may arise by it. This does not mean that new genes came into being; the genes present were recombined in as far as the total potential variability of the hybrids permitted. Only new characters arose, which never showed themselves in the species without crossing.

Dr. Bonhote in England crossed the Egyptian house-rat, *Mus alexandrinus*, a gray agouti rat, with rather

short tail and dark belly, with the Egyptian tree-rat, *Mus tectorum,* a fulvous-agouti smaller rat with a long tail and white underparts, sharply demarcated. The young were all like tectorum. These animals, on being mated *inter se,* gave some dark-bellied young, and from the mating of two "tectorum" young he obtained, together with some dark-bellied and white-bellied agoutis, a few orange yellow rats. At this stage we took over his animals. Through the excessive zeal of the French custom-officers, who feared that the animals might carry malaria(!), they were returned from Dieppe to London, and most of them died on the way, including all the yellow ones. When finally the rats reached us in Verrieres, we obtained only a few white-bellied animals.

White-bellied female no. 13 finally mated in the big room with a black French *Mus rattus* male, after having killed a great many males in cages. The hybrids were black and had very long tails. We lost a good many in transporting the animals from France to Holland.

One of the white-bellied agouti rats obtained, tree-footed number 17, was mated to two black hybrid females, 24 and 25, and with tectorum female no. 19. From the mating of 17 with 24 we obtained twenty young, of which seven were blacks, seven white-bellied agouti (like tectorum), five yellows and a few pearl gray. Among the blacks one had a white tail tip, and one of the white-bellied agoutis had also a white tip to the tail. Three of the gray-bellied agoutis were waltzers. These animals behave exactly like waltzing mice. They run around in small circles with amazing rapidity, and they are unable to climb. But whereas waltzing mice are less viable than their normal litter brothers, the waltzing rats are as vigorous as normals. And whereas waltzing mice are congenitally deaf, our waltzing rats can hear perfectly normally.

From the mating of male 17 with female 25 we obtained seventeen young, seven blacks, of which one waltzer and one white-throated, seven white-bellied agouti, two gray-

bellied agouti, and one agouti with lemon-yellow belly. As females 24 and 25 were litter sisters, with the same parentage, we may be allowed, for the present discussion, to add their young together. There were 37 young, of which 14 were blacks and 17 agoutis (expected 15.5 and 15.5). Of the 37, five were yellow, one pearl gray, two with white tail tip, one white-throated, four waltzers, and one yellow-bellied, all of which are animals with totally new characters.

We can easily explain the origin of the new characters as follows. If both parent species possess a gene, which by its presence or absence makes the difference between a normal and a waltzer, or in other words, if to be normal a rat's germ must at least possess either Y or Z, the hybrids, which are impure for Y as well as for Z, having inherited Y from one and Z from the other parent, will produce one germ-cell in every four, from which both Y and Z are lacking. Therefore such hybrids will produce, when mated among themselves, fifteen normal young and one waltzer in every sixteen. If we expect the same reasoning to hold good for a number of new recessive characters, which are displayed by neither of the parents, so that animals lacking W and X will be yellows, others, lacking U and V, will have white-tipped tails, we should in our case expect to find among our thirty-seven young, two to three (2.312) with the new character in every case. In reality we found yellow five, pearly gray one, white tail tip two, white throat one, waltzers four, yellow belly one, that is 2.33 on the average.

These numbers make it clear that we are not dealing with a sort of period of mutation; it was easy to see that the new types were already given in the genotype of the three species crossed.

Female no. 24 later was mated back to her son no. 95. From this mating we obtained among a number of normal rats, one chocolate and two pearl-gray young. Later we obtained a cinnamon agouti rat, that is to say an animal that probably stands genotypically in a relation to agouti,

as chocolate to black. It is to be remembered that in this group black is dominant to agouti. In all we obtained six wholly new characters from our matings, clearly as the result of the absence of two genes in every instance.

Matings of white-bellied animals with gray-bellied gave either only white-bellied or a minority of gray-bellied in F_2. Gray-bellied rats clearly have a gene less than white-bellied. This is the same result which Morgan obtained in his work with animals of this group. Black was dominant over agouti and clearly there were two kinds of blacks, with or without the gene which makes the difference between white-bellied and gray-bellied agoutis. We never obtained white-bellied black ones. But the blacks with the gene under discussion had a much more deeply black color, very often with a green or a violet sheen. We obtained yellow-bellied yellows, and, just as in the agouti series, white belly was dominant over yellow. Male 28 and female 34, both white-bellied yellows, gave three white-bellied and one yellow-bellied young. Our chocolate and cinnamon rats died on the steamer bringing them to Java. The character white tail tip proved to be recessive. We obtained pearl-gray young and yellows from matings between yellows and pearl grays, but yellows never produced pearl grays. Two agouti animals sometimes produced yellows, but never pearl gray. These were only obtained when one parent was either pearl gray or black. In other words, the factors which produce the difference between black and agouti animals are the same which make the difference between pearl gray and yellow.

Our new rats, waltzers, and animals with new colors, such as they are can not be called species. We can make species out of them by continuing the breed. If we sell a number of animals of one color to rat fanciers, and they get sufficiently enthusiastic over them to provide classes for them at pet-shows, we will be justified in calling such a breed a domestic species.

We saw that in our experiments with rats no new dominant characters originated, unless we want to call

the colored sheen on certain black ones by that name. In every instance there appeared new recessive characters. For every one of them we could see that crossing, recombination of genes, was the cause, not loss-mutation. But it becomes clear that it is very difficult to be sure that apparent cases of loss-mutation are not due to recombination, unless the number of young in the generation in which the novelty appears is rather large. If we mate a species A to a species B, and some yellow or long-haired or albino animals are produced in F_2 we are rather sure that recombination and not loss of genes causes the novel form, even if the number of young is too small to know whether the new character was found in one animal among every four or among sixteen. But if we mate two animals belonging to one single species, and it happens that each possesses a gene which the other lacks, the two genes having equal influence on the development but of such a nature that animals lacking both are albinos, or yellows, the production of a few animals with new recessive character may easily be looked upon as mutation. In such an instance, it will be found that the two animals which produced the heterozygote who gave the aberrant young would be found to be homozygous in respect to the presence of "the" factor. For if we mate an animal having YY to the new form yyzz, all the young will be dark, and none albino. Conversely, if we mate the ZZ parent to the albino, it will also be found to be homozygous, all the young will be colored. In other words, test mating will in certain instances be insufficient proof for the occurrence of a loss-mutation.

In the days when we talked about "unit-characters" and the factors which "determined" unit characters, it was commonly held that crossing in the widest sense, mating of forms with diverse genotype could not count for very much in evolution, as it could only recombine existing characters and not create new ones. We have since learned to look upon the genes as upon things which help with other factors in the development to make an organism develop, and we now know that the action

of genes upon what were called "unit-characters," is a very indirect one. We now know that new characters may certainly come into being through recombination of genes. Recombination may result in the origin of new recessive characters, and this process may look very much like loss-mutation. And crossing may result in the origin of new dominant characters, color in chickens, in rabbits, extra toes in chickens, and this process will look very much like positive mutation, the creation of a gene out of nothing. If we except Œnothera species, dividing the organic world into animals, plants and Œnotheras, for as long as no solution is found for the baffling delayed and abnormal segregation in Œnothera hybrids, we may sum up as follows:

Evolution is the result of a combination of all those causes which heighten variability and which limit it.

The only cause for inheritable variability in multicellular organisms which can be of any account in evolution is mating between individuals of unequal genotype, crossing in the widest sense (Amphimixis).

All those causes which tend to reduce the potential variability of a group of organisms tend to make varieties or species of these groups. Such causes are isolation, migration, adaptation, selection and *especially* the fact that, either periodically or regularly, the number of individuals of one generation is very much smaller than that of the preceding one. This cause of purification of the type, which we see in operation everywhere (think of the numbers of house-flies a year in the last and first generations), operates quite regardless of adaptation or fitness. To this cause working upon variation may be ascribed numerous characteristics for which we can invent no earthly use, and for which nevertheless species are pure.

Whereas species and varieties are realities, systematic division of the organic world into groups of higher magnitude is wholly arbitrary, and may without any doubt be arranged to suit the capacity of museum cupboards.

DIFFERENTIATION BY SEGREGATION AND ENVIRONMENT IN THE DEVELOPING ORGANISM[1]

DR. VERA DANCHAKOFF

Biological investigations in the twentieth century have markedly strengthened the belief in the specificity of different kinds of living matter. Paleontology has shown the existence of organisms which have retained their specificity during millions of years: specific germplasma has carried through ages specific characters. On the other hand discoveries in the world of microorganisms have shown, that even their simplest forms are characterized if not always by specific organization, at least by definite metabolism and other biological qualities which imply a specificity of their constitution.

I shall not discuss the problem of genus specificity. My subject is limited to the specificity of certain tissues and cells found in the organism, the final development of which results in the symbiosis of differently organized tissues. The problem whether the relations of these different tissues is definitely determined by their specificity, or whether there exist in the organism plastic factors which from a homogeneous cell material may mould differently organized products is still unsettled. The solution of this problem would be greatly advanced, if the results of experimental and descriptive histogenesis received due consideration. Though the microscope can indeed not distinguish between various colloidal solutions, it might and does give data of definite biological significance.

Different genera and species show under the microscope a different structure of their building stones—the

[1] From the Anatomical Laboratory of Columbia University. Read before the Section of Biology, New York Academy of Sciences, April 9, 1917.

cells, and most conspicuously in their chromosome-complexes. The specificity manifested by genus and species, whether it is centered in specific proteins of the cytoplasm or in specific molecules of the chromosomes, forms one great chapter of the specificity problem, while the specificity of different tissues and cells is another.

It is often stated that the different tissues and cells of an individual of a given species, all have identical chromosome-complexes. If the chromosomes are considered identical in all the cells of an individual, they can not be regarded as responsible for the specificity of his tissues. They can place no restriction upon a wide range of permutability between the various cells in the organism, can put no restraint upon unlimited regeneration or impede the perpetual proliferation of any type of cells. The assumption of equality of chromosome-complexes in different tissues and of their invariability excludes them from the range of possible carriers of the specificity of tissues and is usually associated with the belief that the specificity of tissues is brought about by segregation of cytoplasmic materials during development. The possibility is also considered, that environment may act as differential factor.

Of these two latter factors the segregation of cytoplasmic materials in the early stage of development leads to the formation of large cell groups (germ-layers, anlages of organs), the differential characters of which are believed to be determined by the presence of definite cytoplasmic materials, transferred to them from the cytoplasm of the ovum. The differentiation brought about by segregation is regarded as irreversible and though the cells of the germ-layers show a great plasticity in their response to different factors, there is a well-marked limitation of their potencies, if compared with the first blastomeres. It is believed, however, that the segregation does not affect the chromosomes and produces merely a differential distribution of the cytoplasmic constituents of the ovum among the resulting cell groups. It has been

recently shown that at least the embryonic mesenchymal cells have an unlimited power of regeneration and therefore can be considered potentially immortal.

In passing I should like to point out that the uninterrupted synthesis of the chromatin during cell proliferation may be secondarily influenced by the differences in the cytoplasm thus acquired. The assumption of invariability on the part of the chromosome-complexes would imply a further assumption of persistence in the cytoplasm at least of some unchanged metabolic processes identical for all cells, to which the synthesis of identical chromatin could be referred.

Differentiation by segregation is a fact proved experimentally and many striking examples of segregation of various cytoplasmic materials during cleavage are found in Wilson's and Conklin's work. As result of segregation a number of cell groups appear. The groups are different, but the cells of each of them are similar. The various cytoplasmic substances distributed to the cell groups are specific, can not be built up by cells, which do not contain them and influence the further development of the cells in a definite manner.

These groups of cells proliferate and differentiate, giving rise to a number of specific organs, tissues and cells. Does a further segregation of definite substances continue at the time of the final specialization of tissues, are the cell potentialities gradually narrowed by further differential distribution of cytoplasmic constituents, and finally rendered univalent and irreversible? Leaving aside the question as to how specific tissues arise from specific anlages, Loeb in his last book, an important and stimulating publication, adopts the view shared also by Stockard, of the specificity of anlages in the organism. *The anlages are,* in Loeb's conception, "*destined to give rise to definite organs.*" He considers "the formation of the various organs of the body, as being due to the development of *specific cells* in definite locations in the organism, *which will grow out into definite organs, no*

matter into which part of the organism they are transplanted." This assumption may apply to a number of developmental processes in the organism, but the statement is only part of the truth. Years of study of the loose mesenchyme and of the differential processes, observed in this tissue, have yielded a few results, which most decidedly do not harmonize with the generalization above quoted.

The loose mesenchyme, which appears in the early stages, is characterized by its ubiquity and by lack of obvious special function, if its mere presence between other organs has not to be considered as function. The mesenchyme is a syncytium of similar cells, the structure and most probably the metabolism of which is little if at all changed, while the cells remain as constituent parts of the syncytium. Influences of local origin which might change the metabolism of some of the cells are inhibited by continuous unimpeded flow and intermixture of substances in the undivided bodies of the cells.

Cells of the loose mesenchyme become isolated from the syncytium in many parts of the organism. This process of isolation is diffuse, in some parts of the organism it affects merely a small number of cells, in others it is displayed with great intensity. Scattered free cells or large groups of them are formed. The cause of such *isolation?* If it is not possible to formulate it in positive terms, at least it can be stated, that *it does not depend upon predestination,* centered in the syncytium itself, since isolation of cells from a mesenchymal syncytium can be greatly intensified experimentally. Large groups of free cells develop in the embryo after certain grafts on its allantois in regions in which normally the cells would retain their syncytial connections.

The free ameboid cells isolated from the mesenchymal syncytium differ from the cells of their maternal basis in many respects. Their metabolism is no longer controlled and regulated by the metabolism of the whole colony of mesenchymal tissue. Isolated they are very active, grow

intensely, frequently divide and their structure undergoes rapidly a series of changes which are not always identical and which transform them into various blood cells. Do these various changes exclusively depend upon the physicochemical constitution of the cells, in other words are they predestined, will each of these cells grow into a definite unit, no matter to what condition it is subjected? Is the group of ameboid, morphologically similar cells freed from the mesenchymal syncytium still formed by a number of species cells, the characteristics of which consist if not in a discernible structure, yet in an inherent necessity to develop along definite lines?

A series of investigations, some of them my own, have pointed to the group of the free ameboid cells as the mother cells of various blood elements. In regions where the isolation is merely occasional, scattered wandering cells arise. In regions where the isolation of free cells is intense, so-called anlages of hematopoietic organs develop. The first stages of development of various hematopoietic organs were found to be much alike and the continuous differentiation of the various blood cells throughout life was shown to have for its starting point a cell, the structure of which is similar to that of the ameboid cell, which arise from the mesenchyme.

Moreover, it was observed that there existed an invariable *association between the development of the mother cell into a definite blood cell and definite environmental conditions*, viz., if left in the spaces amongst mesenchymal cells the free ameboid cell develops into a granuloblast, especially in the vicinity of thin walled vessels; if surrounded by endothelial walls and subjected to intravascular conditions it develops into an erythroblast. This association has been established in the hematopoiesis of birds, reptiles, amphibia and certain fishes. The association between differentiation of the stem cell and environment on account of the regularity with which it was observed suggested to me the idea, that it was more than mere coincidence, and that possibly environment contained the differential fac-

tors, which from a homogeneous cell material moulded different products.

On the basis of descriptive histogenetic studies it seemed plausible to admit that environment can modify isolated cells; that the metabolic processes of the cells are the resultant of their physico-chemical constitution plus physico-chemical conditions of the environment (of course hormons, enzymes and so forth are included in the environment) and do not depend exclusively upon their physico-chemical constitution; that different substances arise in the cell-body (hemoglobin, various specific granules) in polyvalent cells as result of changes, determined by differences in the environment. The existence of cells endowed with various potencies has in consequence been largely admitted. The specificity of the various mature blood cells would thus be brought about by factors extrinsic to the stem cells.

These conclusions are based on facts established by descriptive histogenetic studies. Experimental proofs are beginning to accumulate which soon will leave no doubt of the validity of these conclusions. The existence of polyvalent cells would be proved, if, for example, hemoblasts subjected to various conditions would undergo various differentiation. If stem cells from within the vessels were transferred into the spaces between the mesenchymal cells and here instead of developing into erythroblasts, differentiated into granuloblasts (these experiments are under way) the stem cells within the vessels would be proved to be polyvalent. The same applies to other blood cells. As recently shown, spleenic follicles, the cells of which normally differentiate into small lymphocytes, if grafted on the chick allantois resolve themselves into numerous hemoblasts, which finally undergo a granuloblastic differentiation and give typical granular leucocytes. Thus the results of the histogenetic studies by experimental method entail the recognition in the embryo and in the adult organism of tissues and cells, which have not been fully differentiated and remain polyvalent.

It is the polyvalent cells which are the source of the wide range of regeneration encountered, particularly in the lower animals. It is astonishing to see how readily students of differentiation and specificity reconcile the extensive regeneration observed in many organisms with the belief in the specificity of the anlages of organs. Driesch has shown that gills excised from an Ascidian can regenerate a whole animal with heart, intestine and stolon. If in the particular case the anlages of the gills and the gills themselves were built of specific cells, the results of the experiment would be inconsistent. How could heart, intestine and stolon regenerate from the gills if the cells of the gills were not endowed with various potencies; if specific, they would grow only into the same tissue under all conditions. On what other basis could the experiments of Child's be explained, in which cells of a definite segment in the *Planaria* will regenerate a head or a tail, according to whether it formed the anterior or the posterior part of the piece cut out from the worm? The very fact that different specific structures may be regenerated at the expense of one common source, as, for example, heart and intestine from a gill or erythrocytes, granulocytes and small lymphocytes from hemoblasts, implies the polyvalency of their common source.

It is known, indeed, that environment can educe new qualities in the organism, but they usually subsist only while the specific conditions are present, and are lost if the organism is transferred to another environment. Such changes are not specific. The changes revealed by the freed mesenchymal cells, which result in the formation of mature blood cells, would only then be called specific, if they were retained by generations of their descendants under different conditions. An indifferent hemoblast within the vessels is soon transformed into an erythroblast, which shows in its cytoplasm the first traces of hemoglobin. Is the erythroblast a definitively specific cell, univalent and no longer capable of heteroplastic differentiation in new environment? New environmental condi-

tions for an erythroblast can be found in the organism outside the vessels, where hemoblasts develop into granuloblasts or small lymphocytes. If transplanted outside the vessels, the erythroblasts still developed further into analogous cells, this would mean that the changes which inside the vessels have transformed a polyvalent hemoblast into an erythroblast are irreversible (at least in the organism), that they have narrowed the potencies of the erythroblast in comparison with its mother cell and have rendered it specific, *i. e.* univalent and irreversible in its metabolism. Positive results from such experiment, could they be attained, would be of great value; they would prove that definite factors encountered in the normal organism outside of a cell call forth such changes as would be transmitted by the cell to its daughter cells even if the differential factors had no longer direct influence upon them.

The arrangement of such experiments offers however insuperable difficulties. Hemoblasts or mesenchymal cells can be transplanted, for there are stages in the hematopoiesis of the yolk-sac, in which capillaries are distended exclusively by hemoblasts and at this time they can be transferred into the spaces between the mesenchymal cells. It would be hardly possible to pick out from within the vessels erythroblasts, in which hemoglobin had already begun to develop, but which still were capable of proliferation. Most fortunately the required experiment has been carried out in a series of allantois by nature herself.

The grafting on the allantois which I used in my recent work is often accompanied by an extensive edema in the mesenchyme, which also affected the endothelium of the vessels. At the time of grafting (seventh to eighth day of incubation) the vessels contain numerous young erythroblasts, which after grafting become particularly numerous. The loosening of the vascular wall made it possible for a number of erythroblasts to escape from within the vessels. As a result of these conditions large

groups of cells appeared in the spaces between the mesenchymal cells, which already had begun their erythroblastic differentiation, while within the vessels. These cells, now outside the vessels, proliferate and continue their differentiation into erythroblasts, and their cytoplasm is gradually transformed into or substituted by homogeneous hemoglobinic substance.

The changes undergone by a polyvalent hemoblast within the vessels are thus no longer reversible outside of them. The differentiation determined by environmental conditions has been rendered specific, *i. e.*, univalent and irreversible. The specificity of tissue and cells can not therefore be the result alone of segregation of different cytoplasmic materials during cleavage. The process of segregation, of course, transfers different materials to different cell groups, the presence of which impedes their permutability, but these cell groups are still polyvalent and may, under various conditions, undergo various development.

The relations between these cell groups, the structures, effected by them, the different products of their metabolism, form the external factors of the environment which gradually render the cells of a polyvalent group specific, univalent and irreversible in their potencies. This specificity is transmitted by mother cells to their daughter cells irrespectively of the environmental conditions, to which they are subjected.

A few words concerning the structural changes of the cells during their definitive specialization. Differentiation during cleavage is effected by transmission of different cytoplasmic materials to different cell groups. What kind of changes in the cell structure are induced by the external factors of the environment? Compare the structure of the mature univalent blood cells with that of their mother cells in the stage of a hemoblast. Cytoplasm, structure of the resting nucleus, chromosome-complexes during mitosis, as demonstrated by our microscopical preparation, have undergone such fundamental changes,

as to have required thorough and detailed investigations in order to establish their reciprocal relationship. The size of the cells makes difficult a detailed study of the changes in the chromosomes, and they require further investigation, nevertheless the possibility of distinguishing different types of chromosome-complexes in different cells is not to be overlooked; it is easy to identify, for example, in the thymus entodermal cells, hemoblasts and small lymphocytes, during mitosis by their chromosome-complexes. The assumption of invariability on the part of the chromosome-complexes in the somatic cells requires some qualification. The chromosomes of a cell and the cytoplasm together embody specificity. Changes in both may transform the cell so completely as to deprive it of its faculty of proliferation. Erythrocytes and leucocytes in the blood cell series afford examples of such final modifications which have been gradually determined at least in part by the external factors of the environment.

A METHOD OF NUMBERING PLANTS IN PEDIGREE CULTURES[1]

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ABOUT fifteen years ago, Dr. H. J. Webber (1906, p. 308) introduced into the plant-breeding work of the United States Department of Agriculture a simple and convenient method of pedigree numbering. This method has three essential features: (1) the use of an initial "series number" for each hereditary line or group of lines, the sets of series for different crops being numbered separately; (2) the use of letters for particular parental combinations in a hybrid series; (3) the use of numbers separated by dashes to designate individuals of successive generations.

For example, with cotton, Series 1 might represent selection for longer lint, within the variety Columbia; 1–1, 1–2, etc., would then designate the plants first selected (P_1 generation), and 1–1–5 would designate a plant of the second or F_1 generation.

Similarly, Series 2 might represent a cross between two varieties (for example, Columbia ♀ × Truitt ♂), and Series 3 the reciprocal of this cross. Each combination of "individuals" (plants, branches, or single flowers, as desired) within a hybrid series is represented by a letter, as in 2A, 2D. The F_1 plants are then designated by numbers written after the letters, as in 2A1, 2D6, and the numbers for later generations follow dashes exactly as in a non-hybrid series (for example, 2A1–5). The use of letters thus characterizes hybrid series.

This system of numbering, then, uses simple linear pedi-

[1] Paper No. 40, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station. Riverside, California.

grees for all cases, beginning a new pedigree whenever two lines of descent are combined by crossing.[2] It is adapted to self-fertile organisms, and its convenience and usefulness obviously increase with the frequency of selfing. Pearl's (1915) "system of recording types of mating," on the other hand, is especially adapted to diœcious and self-sterile organisms, and in general its usefulness for recording or presenting data increases with the frequency of crossing.

Several systems similar to Webber's have been described in recent genetical papers; these schemes usually differ mainly in the method of indicating the "series" or "families." In Jennings' (1916, p. 415) system for asexual reproduction with the animal Difflugia, for instance, each "family" is a pure line descended from one selected individual; each pedigree number, consequently, begins with a simple number assigned to the P_1 individual. Here we have the simplest possible form of pedigree numbering. With the higher plants, however, we need some special provision for cases of crossing, and also some method of distinguishing asexually produced individuals from those produced sexually.

Belling (1914, pp. 309–10),[3] for Stizolobium crosses, uses for each series the initials of the common names of the parent species (*e. g.*, VL for velvet bean ♀ × Lyon bean ♂); this is practicable because the crosses involve only a small number of named forms, themselves appearing practically constant. For the same reasons, the P_1 plants, and usually the F_1 also, are not individually indicated in published pedigrees; VL3 is an F_1 plant, and VL3–7 or VL–7 an F_2 plant. The obvious result of this procedure is a gain in both clearness and brevity of presentation.

Hayes and East (1915, p. 3) use for maize crosses a scheme that resembles Belling's in its direct indication of the parentage of hybrids. "The various races were given

[2] Except in cases where vicinism is undiscovered or ignored.

[3] Or see Fla. Agr. Exp. Sta. Report for year 1913, 1914 or 1915.

different numbers as No. 10 flour corn and No. 5 flint corn.'' Then the F_1 hybrids would be designated, for example: (10 × 5)–1, (10 × 5)–2, (5 × 10)–1, etc. If, however, the individual P_1 plants are regularly indicated in the series numbers, these numbers tend to become unwieldy, particularly if the P_1 plants themselves have somewhat lengthy pedigrees. In cases where this objection does not apply, and where Belling's method is inadequate, Hayes and East's scheme has some advantage in the immediate significance of the series numbers of hybrids.

Of the systems described, Webber's seems most generally applicable to work with the higher plants, though the others may be somewhat more convenient in certain cases. I wish to present several additions to that system, designed in part to provide for somatic variation and polyembryony.

First, capital letters may be used, as is often done, for various special purposes. For instance, letters are sometimes added to distinguish particular types; Shull (1908, p. 60), in describing his system of pedigree records, restricts letters (aside from Roman numerals) to this use, and Hayes and East (1915, p. 4) use them to designate floury and flint-like types of maize kernel. When temporary lot numbers are used, ''L'' prefixed to these numbers will distinguish them from any others. Again, Arabic numerals preceded by ''R'' seem more convenient than Roman numerals as used by Shull (1908, p. 60) to designate rows in field cultures—giving, for instance, R1, 3 (row 1, plant 3) for I, 3, and R28, 26 for XXVIII, 26. I would suggest that capital letters, and capitals only, be employed for such miscellaneous purposes.

Second, small letters may be used for the indication of parts of individuals, whether the parts remain attached or are separated in vegetative propagation.[4] Then, ''–51a'' in a Citrus pedigree will indicate a particular part of tree 51 in a certain generation of that series—

[4] This is to replace a method of indicating parts of individuals by means of fractions.

perhaps a part permanently marked, and noted in the records, because of some somatic mutation. In the same way, a small letter affixed to any individual designation of a plant not in a pedigree culture, such as, for instance, the number of a tree in a field experiment, may be used to indicate a particular branch. A small letter used alone will indicate a "generation" of vegetative propagation; for example, in the case mentioned above, "–51a–a" and "–51a–b" would be trees budded from the mutant branch "–51a."[5]

Third, small letters affixed to the series number may be used to designate individuals of the first (or P_1) generation of any series, *whether hybrid or not*.[6] The definition of *individual* would necessarily be determined and recorded for each type of work; where desired, the "individual" may be a branch, or even a single bud or fruit. Thus, in my work with Citrus, a letter is assigned to each self-pollinated branch giving seedlings. For example, series 28 consists of descendants of selfed Valencia orange; "28a," then, indicates one bagged branch of Valencia, "28b" another branch, etc. The F_1 progeny will be 28a–11, 28a–12, 28b–32, etc.

For a hybrid series, on this plan, two letters are used to designate the two parents, the letters being independent of those used in the corresponding non-hybrid series. For example, my Citrus series 27 represents Valencia orange ♀ × Imperial pomelo ♂, and "27aa" designates a cross between two particular branches. 27aa–21, then, will be an F_1 hybrid.

Similarly, in my work with Matthiola mutants, series 23 indicates the cross Snowflake ("normal") type ♀ × Slender type ♂. "23ea" designates the combination of

[5] The complete pedigree numbers might be 28a–32–51a–a and 28a–32–51a–b (see below).

[6] In cases where the initial parents of a series are selected from larger groups requiring designation of individuals, the plants of these larger groups may be given temporary numbers. For example, my Raphanus series 14 began with plants from one lot of commercial seed of one variety, numbered 14, 1; 14, 2; 14, 3; etc. In the next generation, 14, 3 became 14a, and 14, 16 became 14b.

two particular individual *plants*, not of two particular *branches* as with Citrus.

Thus with Matthiola a series starts with the crossing, in a given direction, of two apparently uniform *seed-reproduced types*, while with Citrus a series starts with the crossing of two *clons*. In another case it might be desirable to ignore some evident genetic variation in features not being studied, and to base the delimitation of the series on genetic differences of seed-reproduced P_1 individuals in some particular feature under consideration (*e. g.*, in color characters).

Fourth, in cases where polyembryony is to be expected nine numbers (1 to 9), instead of one, may be given, so far as needed, to the product of each seed. Thus, with the Citrus cross mentioned above, the first seed will be assigned the numbers 27aa–11 to 27aa–19, the second seed the numbers 27aa–21 to 27aa–29, etc.[7] The actual plants from the first three seeds are therefore designated as follows:

Seed No. 1 (2 plants), 27aa–11 and 27aa–12.
Seed No. 2 (2 plants), 27aa–21 and 27aa–22.
Seed No. 3 (1 plant), 27aa–31.

If, then, two trees are budded from tree 27aa–31, these will be 27aa–31–a and 27aa–31–b.

It may be objected that this treatment of polyembryony gives misleading numbers to plants from adventitious embryos. It may, however, be impossible in many cases to differentiate positively these asexually produced progeny before maturity, if at all without progeny tests, and the probable inapplicability of some of the numbers must be kept in mind. Where, as with crosses of *Citrus trifoliata* with Eucitrus species, the strictly maternal individuals can be separated at an early stage, they can be discarded; or, if such plants are to be kept, and their exact origin indicated, letters may be used, thus:

43aa–11 (true hybrid).
43aa–1a (for 43aa–12) } (plants from adventitious or
43aa–1b (for 43aa–13) } asexually produced embryos).

[7] The seeds themselves might be called 27aa–10, 27aa–20, etc.

By thus inserting letters in the hybrid number, to designate the second and third plants, we indicate the vegetative origin of these plants, and also their quasi-sib relation to the true hybrid.

Open pollination, or lack of protection of flowers in general, can be indicated in the numbers, if this seems desirable, by underscoring the proper letter or number,—the use of two letters with the series number for hybrids making it possible to do this with the pollen parent as well as with the seed parent.

An incidental advantage over the original plan results from the use of letters with the series number in the way here indicated. This consists in the fact that the numbers for hybrid and non-hybrid series are thus made symmetrical in relation to the number of generations involved. As an illustration we may take the case of the Matthiola cross mentioned above. This cross was made in 1914, and the hybrid seeds were planted, together with seeds from selfed parents of the same types, in 1915 and 1916. Corresponding to the F_1 hybrids 23ea–1, etc., we have the F_1 selfed progeny 19a–1, etc., from a Snowflake parent, and 25c–1, etc., from a Slender parent, while by the original method these three numbers might be 23G1, 19–1–1, and 25–3–1.

As will readily be seen, this scheme is elastic; if the five main features stated above are adopted, different workers may add, modify, or omit various details, and still use numbers intelligible to each other with little or no special explanation. Further, the method of designating series and initial individuals is essentially independent of the other features suggested, so that any of the latter features may be adopted without the former.

All the schemes so far discussed give *cumulative* numbers, which include the whole pedigree from the P_1 individuals down. Shull (1908, pp. 59–64) has described a non-cumulative system, in which individuals have only temporary numbers (in the field depending on row and position in row) until selected as parents. Each parent

is given the number of the notebook page on which its progeny are to be described, preceded by two figures indicating the year in which those progeny are grown; *e. g.*, 06230 is a parent whose progeny are grown in 1906 and described on p. 230 of the 1906 notebook. Only parental and grandparental numbers (*e. g.*, 0557.230 for the case just mentioned) are shown at the head of each notebook page, but these numbers permit ready reconstruction of pedigrees from the pages indicated. The labels bear only the parental number.

In Shull's scheme, then, there is no cumulation beyond the second generation, even in the numbers as written in the notebook. The numbers would include 4 to 8 figures (*e. g.*, 083.7, or 05157.230) in the notebook and 3 to 5 figures (*e. g.*, 097, or 06230) on labels. In the scheme here suggested there is a continuous cumulation; a plant of the second or F_1 generation is usually represented by 3 to 7 letters and figures (*e. g.*, 5b–8, or 32ba–251), and after from 2 to 4 more generations the numbers begin to be decidedly unwieldy. The greater inconvenience and danger of error in copying these larger numbers would seem, however, to be largely offset by the growing familiarity to the worker of the earlier part of a pedigree, and by the identity of the temporary and permanent designations of individuals. Further, the page-to-family feature would be inconvenient in some cases, where several or many actual pages are given to one progeny lot and so would require the same number.

With a cumulative system, the simple device of using temporary yearly lot numbers on individual labels will obviate the necessity of writing a long pedigree number for each plant. If the full number is written in the notebook and on each lot label, together with the lot number (*e. g.*, L1 = 16aa–6–3–44–18–3), and the latter alone on the individual labels if these are used, much work can be saved. In an extreme case, the parents belonging to a given series which are included in a given culture can be

arbitrarily made the initial (P_1) individuals of a new series.

The system here presented has been used for two or three seasons' work with the two types of hybridization mentioned above, with Raphanus hybridization beginning with highly heterozygous material, and with pure-line breeding of the tepary bean (Phaseolus). It appears, so far, to be adequate and generally satisfactory for all these types of work.

SUMMARY

This paper describes a system of pedigree numbering adapted to various types of genetical work with the higher plants.

Other similar systems secure greater brevity or clearness in certain cases, but are usually of less general applicability; the main differences relate to the designation of series and their initial individuals.

This system provides for polyembryony and somatic variation, and permits of the addition of various other refinements in cases where they may be needed. The basic scheme is perhaps as simple and convenient as is consistent with use for all purposes without change in essential features.

In this and similar systems, the numbers are cumulative; Shull's non-cumulative system has both advantages and disadvantages in comparison.

To summarize the most essential features of the proposed method: (1) the series for a given plant (genus or species) are numbered consecutively; (2) the initial "individuals," as defined in the records, are denoted by small letters affixed to the series numbers; (3) in each following generation the individuals are numbered (if sexually produced) or lettered (if asexually produced), an affixed letter indicating a particular part of an individual; (4) reproduction or propagation is always indicated by a

dash;[8] (5) capital letters are employed for miscellaneous special uses.

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[8] Of course, a period may be used, as is done by Jennings (1916) with Difflugia, with a saving of space but possibly a greater liability to error in copying.

SYNCHRONISM AND SYNCHRONIC RHYTHM IN THE BEHAVIOR OF CERTAIN CREATURES

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IT is a matter of common experience to observe instances of synchronous behavior and expression among creatures. In such instances an entire group of creatures may react simultaneously to the same external stimulus. A flock of birds will arise from the ground and dash away at the first signal of danger, or a school of fish will swerve as a unit from a stick pushed toward them. In the same way certain frogs in a pool may be started into a brief, explosive chorus of simultaneous croaking by the notes of a single individual. After a brief period during which all have expressed themselves, silence ensues until the next singing-reflex is unlocked by the croaking of another individual. In New England I have heard the wood frog, or so-called clucking frog (*Rana sylvatica*) give rise to just such outbursts of simultaneous clucking, started either by the frogs themselves or by my giving an imitation of their notes during an interval of silence. This habit of singing in concert is not unusual among certain species of frogs, and is mentioned by D. D. Cunningham (1903) in his excellent book, "Some Indian Friends and Acquaintances." He says:

> Such utterances recur several times in succession; a short pause follows and then the conversation begins again. The curious thing is that all the performers seated in one patch of swamp should have such a tendency to synchronous action that periods of total silence alternate with those of general uproar. The phenomenon is parallel to that of the synchronous luminosity that sometimes occurs so markedly in groups of fireflies.

In these instances synchronism is maintained at intervals, but there is no regular rhythmic expression within the group itself. Each frog croaks in its own way, until a perfect babel of noise is produced.

Similar synchronous outbursts of sound also occur among the musical insects. The tendency to respond to the notes of their kind is very strong, and a single singer

may be followed by thousands. Although the seventeen-year cicada rarely sings at night, Hopkins[1] noted a most remarkable nocturnal concert which began with a single singer. He says:

> I was fortunate enough to hear the starting of one of these concerts on a clear, moonlight night in June. One male in an apple tree near the house suddenly called out as if disturbed or frightened. His neighbors in the same tree were thus apparently awakened. One started the familiar song note, which was at once taken up by numbers of other males, and, like the waves from a pebble dropped into still water, the music rapidly spread until it reached the edge of the thick woods, where it was taken up by thousands of singers, and the concert was in as full blast as if it had been the previous day. This continued a few minutes, until all had apparently taken part and the song had reached its highest pitch, when it began to gradually subside, and in a short time silence again prevailed.

In his book, "Bolivia" (1914), Paul Walle (p. 268) speaks of the sudden outbursts of noise in the tropical wilderness as follows:

> As the darkness grows deeper, the silence of the forest is broken by the sound of flight, by sudden rustlings, or by a strident shriek. Immediately the creatures of the forest raise their various cries, the uproar lasts for a moment, and all relapses into a silence in which one still hears, more or less sensibly, the murmur of a million insects.

In such instances where a group of creatures respond simultaneously to the same initial stimulus, we have the simplest case of synchronic behavior. I once witnessed an instance of a similar synchronic behavior in the movements of a colony of plant lice which thickly covered the tip of a twig. While I was watching them, a tiny, parasitic wasp suddenly approached and hovered over the colony preparatory to attacking them. The plant lice became aware of its presence and in an instant the entire colony raised the hind portion of their bodies simultaneously into the air at an angle of about 45° and began waving their hind legs about. This behavior was probably more or less a protective response to the primary stimulus afforded by the presence of the wasp. It is also possible that the reaction, once started in a few individ-

[1] "The Periodical Cicada," by C. L. Marlatt, Bull. No. 14, Div. of Entomology, U. S. Dept. of Agr., 1898, p. 58.

uals, would tend to be transmitted to the entire colony by the mere contact of their bodies.

The same sort of synchronous movement may sometimes be noted in the reactions of the leaves and leaflets of the sensitive plant (*Mimosa pudica*). A sudden shock may cause every leaf of the plant to react synchronously. Sometimes, when the stimulus is applied only to one or two pairs of the basal leaflets, the closing of these and their contact with those beneath them produce a progressive closing movement in all the pairs of leaflets until the terminal ones are reached. In this instance we have a progressive transmission of stimuli quite similar to the transmission of certain automatic reactions which sometimes take place in groups of insects, birds, or animals.

In other instances the reaction to a certain stimulus may involve several similar, instinctive movements producing the simplest form of synchronic rhythm. One interesting instance of this sort of synchronous action has been adduced by H. H. Newman, in *Science*, N. S., for January 12, 1917. Newman found that an enormous colony of "harvestmen," of the genus *Liobunum*, resting beneath an overhanging rock, when disturbed began a rhythmic body movement, raising their bodies up and down at a rate of about three times a second, for a brief period. If the stimulus was set up in a few individuals of the colony, the synchronous body movements spread rapidly over the entire colony. After a time it was found that the reactions became weaker and finally ceased. In this instance it appears that there was a secondary transmission of the stimuli from individual to individual by means of their closely interlocked legs. The writer has noted a similar behavior among the individuals of certain caterpillars which were arranged close together on a twig. These caterpillars, when resting, had the habit of keeping the anterior portion of their bodies raised in the air. If the colony was disturbed, each individual began a synchronous swinging of the free portion of the body from side to side, violently, for a brief period. The primary stimulus could affect all the caterpillars of a group, or a secondary trans-

mission could take place down the twig. Although the swinging was not always in the same direction, a remarkable degree of synchronic movement was brought about.

In those instances where the rhythmic expression of each individual, when set up, continues for long periods of time, as in the case of certain crickets, we have the development of a more complex synchronic rhythm having its origin in the instinctive habit of response, and probably also built up and maintained by the unconscious influences of the regular sounds of the crickets upon one another.

I do not feel the slightest hesitancy in affirming that certain crickets which have the intermittent habit of chirping may build up and maintain a synchronic rhythm under favorable conditions. I refer to the well-known snowy tree cricket (*Œcanthus niveus*), whose synchronous music has been noted and described by many well-known observers and naturalists in this country. I have many times heard the remarkable synchronous chirping of these crickets in New England, and under exceptionally favorable conditions the synchronism has been so marked that waves of solemn, rhythmical music have been produced for long periods of time. This rhythmic music was spoken of as a "slumbrous breathing" by Thoreau. Hawthorne called it an "audible stillness" which, "if moonlight could be heard, it would sound like that." Burroughs called it a "rhythmic beat." McNeill has said of the music of this cricket:

> It is heard only at night and occasionally on cloudy days, but in the latter case it is only an isolated song, and never the full chorus of the night song produced by many wings whose vibrations, in exact unison, produce that characteristic "rhythmic beat," as Burroughs has happily phrased it.

Dolbear, in the AMERICAN NATURALIST for November, 1897, refers to this cricket when he says:

> At night, when great numbers are chirping, the regularity is astonishing, for one may hear all the crickets in a field chirping synchronously, keeping time as if led by the wand of a conductor.

Although the facts may have been slightly overdrawn when Dolbear stated that *all* the crickets in a field were

chirping in unison, Shull was not justified in concluding that the synchronism observed by Dolbear was merely an illusion. If such were the case, it is surprising that so many other excellent observers have also been misled by the same illusion in reporting the music of the snowy tree cricket. For many years I have made a very close study of the stridulations of insects, yet I have noted this synchronous chirping in but one other species of those crickets which possess the intermittent habit of trilling. One may frequently hear great numbers of the common field cricket (*Gryllus pennsylvanicus*) chirping in the fields, but these crickets never show the least tendency to chirp in unison as do the snowy tree crickets. The jumping tree cricket (*Orocharis saltator*) is also an intermittent triller and may sometimes be heard chirping in great numbers in certain copses, yet the "illusion" of synchronous trilling one somehow never experiences. Likewise, there appears to be no tendency whatever for the ground crickets, *Miogryllus saussurei, Nemobius fasciatus,* or *Nemobius ambitiosus,* to chirp in unison. Many other locusts and katydids, such as *Conocephalus ensiger, Conocephalus exiliscanornus,* and the common katydid (*Cyrtophyllus perspicillatus*) produce regular, intermittent notes and stridulate in well-defined colonies, yet so far as I have observed the individuals of a species never show the least tendency to stridulate in unison.

In the south, however, I have heard the tiny tree crickets (*Cyrtoxipha columbiana*) chirping in unison with remarkable precision, producing waves of shrill, rhythmic sound, as in the case of the snowy tree cricket. In this instance great numbers of these crickets were located in the branches of a large evergreen holly tree. The shrill notes of this little cricket are delivered with great regularity, as are the low, solemn chirping notes of the snowy tree cricket. This regularity in the delivery of the chirping of these two crickets is especially striking when compared with the musical efforts of other chirping crickets, such as *Œcanthus angustipennis, Orocharis saltator,* or *Gryllus pennsylvanicus.* This sustained regularity in the rate of chirping of the snowy tree cricket has been noted by

various observers. On the supposition that the rate of chirping of these crickets is entirely a function of temperature, it has even been considered that the crickets may serve as an accurate thermometer. As temperature indicators, however, the crickets can not always be relied upon under all conditions, since wind, humidity, and electrical conditions of the atmosphere preceding thunderstorms, also appear to influence the activities of these insects.

It is now a question as to whether these crickets perceive the rhythm which is so pronounced in the regular sequence of their chirpings. I believe they must, for it is quite evident that they hear and respond to the peculiar rhythmical chirpings of their kind, which have become the common language of the species. If they are able to recognize the notes of their kind, it is reasonable to believe that the rhythmic character, as well as pitch, manner of delivery, and even more subtle tonal differences enter into the recognition.

The rhythmic chirping in unison which oftentimes becomes such a pronounced feature in the music of these crickets takes place only in the evening and appears to depend upon the nice adjustment of certain nocturnal atmospheric relations—moonlight, temperature, humidity, and stillness of the air. Early in the evening, perhaps, a single cricket begins its stridulations which stimulate others to respond, and by degrees the great chirping chorus is augmented. There may be no noticeable synchronism in the chirping at first, but if conditions are favorable, the crickets gradually build up a synchronic rhythm until waves of solemn music are produced by a certain colony.

There seems to be a marked tendency for the individuals of each colony to adopt the rhythmic beat of their particular colony, so that not infrequently a neighboring colony may establish an antiphonal rhythm, with the result that waves of quavering sound swing backward and forward between two neighboring colonies.

How is this synchronal chirping built up and maintained? It is probable that the instinctive habit of re-

sponse tends to bring the chirping of many individuals into a regular synchronism, but I feel, also, that the crickets are somehow unconsciously influenced by one another in their chirpings so that they tend gradually to build up a common synchronic rhythm as the night advances. Once a synchronic rhythm is established, I am inclined to feel that it would be natural for those individuals which began chirping, at first asynchronously, to chirp, sooner or later, in unison with their fellows.

From the tone of certain discussions which have taken place in *Science* for the past several months, it is clearly indicated that the synchronous flashing of fireflies as a reality is somewhat doubted. There is no reason to consider such synchronism "contrary to all natural laws," as Laurent as recently stated in *Science* for January 12, 1917. In the tropics it appears that great numbers of fireflies frequently establish themselves in the crowns of certain trees, and I am not at all ready to deny that under certain conditions such colonies may not flash synchronously, as reported by Cunningham (*loc. cit.*), Shelford, and others. It is unfortunate that in most instances these reports are accompanied by very meager details as to the general behavior of the fireflies in these arboreal colonies. Cuthbert Collingwood, however, in his book, "Rambles of a Naturalist," (1868), pp. 254–255, has given a very careful account of his observations of the synchronous flashing of fireflies, as follows: "At Singapore, and also at Labuan, the little luminous beetle commonly known as the firefly (*Lampyris Sp. Ign.*) is common. When flying singly it shines with an intermittent light which alternates with darkness; but on fine evenings and in favorable (*i. e.*, damp and swampy) locations, they present a very remarkable appearance. Clustered in the foliage of the trees, instead of keeping up an irregular twinkle, every individual shines simultaneously at regular intervals, as though by common impulse; so that their light pulsates, as it were, and the tree is for one moment illuminated by a hundred brilliant points and the next is in almost total darkness. The intervals have about the duration of a second, and during the intermission only one or two re-

main illuminated. To all appearances they are not on the wing at the time but settled upon the tree; for I was able to recognize certain points of light which I especially noticed, and which remained in the same situation with each successive flash. When I disturbed them under such circumstances they flew about at random, each giving out a more rapidly intermittent light. At Labuan, however, I have frequently seen them shine with a steady light as they flew along looking like little, flying stars of the second, or even first, magnitude.'' If there are periods of repose or inactivity during which the insects cease their flashing, it is easy to see how a part of the colony or even all the insects could react simultaneously to the same visual stimulus, such as the sudden flashing of a single individual. If it is the habit of the insects, before again becoming quiescent, to flash several times in succession, following an appropriate stimulus, it is very easy to see how distinct synchronous flashes of light would now and then illuminate a portion of the tree, or even the entire tree. Such synchronic rhythm in the flashing of fireflies would be similar in every way to those instinctive, automatic body movements observed by Newman in the case of the ''harvesters,'' *Liobunum*—the swinging of the pendulum, so to speak, two, three, or more times, as the case may be, following the initial stimulus. As Newman well suggests, it is possible that a transmission of stimuli could even build up and maintain for some time a synchronous flashing in colonies of fireflies in a tree or field. This flashing in unison would parallel the synchronous trilling of the snowy tree crickets, and would not necessarily violate known natural laws governing the instinctive synchronic activities of various creatures.

By selecting two individuals which were evidently chirping in unison, Shull[2] attempted to study the reality of synchronous chirping in a colony of snowy tree crickets by statistical methods. No greater statistical fallacy

[2] Shull, A. F., ''The Stridulation of the Snowy Tree-cricket (*Œcanthus niveus*),'' *Canadian Entomologist*, Vol. 39, 1907.

could be adduced, however, than to attempt to determine the reality of synchronism in a colony of chirping tree-crickets from statistical results obtained for only two crickets of the colony. That synchronous chirping may be a reality, it is not necessary that 100 per cent. of the individuals of a colony chirp exactly in unison. A few crickets chirping asynchronously would not necessarily prove that a synchronism did not exist. As a matter of fact, some individuals always chirp asynchronously, even when the synchronic rhythm is most pronounced. Taking any two crickets from such a colony, the results would depend entirely upon which two crickets were chosen, and either perfect synchronism or absolutte lack of synchronism, would be established.

It is obviously impossible to subject all the individuals of a colony of chirping tree-crickets or a group of flashing fireflies to statistical analysis. Because this is impossible, however, one is not justified in concluding that judgment based upon careful observation is of no value in determining the reality of synchronism. If one hundred men were marching down a street and 75 were in step while 25 were not, judgment alone would establish the fact that a synchronic rhythm existed. This would be quite as true for the chirping of tree-crickets or the flashing of fireflies. As a matter of fact some of the most marvellous discriminations depend upon niceties of judgment alone, and no amount of statistical data would simplify the matter.

I can not yet agree with those who are inclined to believe that the snowy tree-crickets never chirp synchronously, and that it is impossible for fireflies to flash synchronously, especially certain tropical species of fireflies. I will agree, however, that in practically all instances of synchronic rhythm there seems to be no evidence of conscious, intentional imitation, but merely instinctive, reflex, or automatic reactions to certain stimuli, similar in many respects to the unconscious reactions of the leaves and leaflets of the sensitive plant.

SHORTER ARTICLES AND DISCUSSION

SOLID MEDIA FOR REARING DROSOPHILA

BAUMBERGER and Glaser (1) recently described a method of raising the banana fly on transparent solid media, thus enabling the investigator to observe more accurately the rate of growth and metamorphosis and the larval habits of this insect. The medium was made as follows:

> Five or six bananas were mashed up in 500 c.c. of water. This was allowed to infuse on ice over night, after which the liquid was passed through cheese cloth. Powdered agar-agar was then added in the proportion of 1½ gm. to 100 c.c. of the banana infusion. This was then heated until the agar had dissolved. The liquid was then filtered through a thin layer of absorbent cotton into test tubes. The tubes were then plugged, sterilized and slanted in the customary manner.

As pointed out in the above article, one of us (2) had observed that the bacterial growths which always develop on this medium "do not seem to harm the larvæ" and the mold which sometimes appears "is usually destroyed by the larvæ just as soon as they hatch." This question was further investigated and it was found (2) that the prinicpal food of *Drosophila* is yeast and the flies can not develop on banana which is kept free from microorganisms.[1] Delcourt and Guyenot (3) had previously (unknown to the author) published similar conclusions and Loeb and Northrop (4) had confirmed them shortly before the author's report (2) was sent to press.

It is therefore very well known that the food of *Drosophila* is yeast and the prime necessity of any medium for rearing this fly must be either abundant food for yeasts to grow upon or the presence of large numbers of yeast cells. If a medium is made of sterile compressed yeast and agar-agar[2] it serves as a perfect food for flies which have been freed from microorganisms; however, if living yeasts develop young larvæ are usually killed. As

[1] Loeb and Northrop (4) raised a few flies on aseptic banana, but all flies were sexually sterile. Guyenot (5) also succeeded in raising a small percentage of flies on sterile potato.

[2] An excellent nontransparent medium consists of yeast cake moistened with water.

adult flies usually carry living yeast cells upon them this medium would be difficult to use for work in heredity. A nutrient medium for yeast would best suit the needs of geneticists. Into such a food the adults or pupæ would carry living yeast cells which would ferment the sugars and produce odorous substances which cause oviposition by the female fly. Larvæ on hatching would spread the yeasts throughout the medium, thus increasing growth and alcoholic fermentation which may prevent the development of injurious microorganisms.

Two media might be suggested for this purpose, viz.: Fermented banana agar and Pasteur's nutrient agar. The former may be prepared by adding two cakes of bread yeast separated in 100 c.c. of water to one dozen mashed bananas, mixing thoroughly and allowing to ferment for twenty-four hours. This material should be pressed through a sugar sack and the liquid resulting thickened by heating with 1.5 gm. agar-agar per 100 c.c. and poured into slant culture tubes, plugged and sterilized.

A perfect nutrient medium for yeast consists of 10 gm. ammonium tartrate, 10 gm. ashes of yeast, 100 gm. rock candy, 1,000 gm. distilled water. This is called Pasteur's culture fluid and can be treated in the same manner as the above but should be sterilized in an Arnold sterilizer for three successive days, as it can not be heated to as high a temperature as the banana without preventing jellation. Pasteur's nutrient solution can be approximated by diluting molasses with three parts of water.

In the two media described above yeasts develop rapidly and furnish abundant food for *Drosophila* larvæ and also produce odors of fermentation which cause the female to oviposit readily. A concentrated food for yeast such as banana flour would probably increase the value of the first medium as this substance is now used for raising yeast in the brewing industry (6).

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BIOCHARACTERS AS SEPARABLE UNITS OF ORGANIC STRUCTURE

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INTRODUCTION

As to the term "character," in the commonly accepted sense of zoology and botany, it has long been known that different characters, large and small, exhibit different rates of evolution and are in this sense "separable" or "independent," while in another sense it has long been known that every character of an organism is correlated or coordinated with every other character in function and adaptation. Our general knowledge of character separability (for the want of a better English term) has been more than confirmed by researches based upon the great discovery of Mendel, namely, that many large as well as minute characters which are closely associated or even blended in the adult organism are very sharply separated from each other in the germ plasm, in such a manner that they may entirely appear or disappear in the crossing, or hybridization of species, subspecies, varieties, races, no less than of individuals. The theoretic separability in heredity of the germinal "determiners" or "factors" of characters is in full accord with the several aspects of separability in evolution previously observed in paleontology, embryology and individual development.

The purpose of this synopsis is to review and bring together some of the noteworthy phenomena of character separability as contrasted with those of interdependence, cooperation, correlation and coordination.

It is probable that this principle of separability of units of function and structure, which has been discovered in so many processes of the organism, will prove to be a universal principle. This principle is more or less a necessary consequence of the unit-cellular structure of the organism; if instead of being composed of cell units, each possessing its peculiar heredity qualities, an organism were composed of fluid or solid masses of different kinds, we can not imagine how it could split up into character units. Yet the cell is probably not the ultimate, or least character unit, which is doubtless a chemicophysical unit. This is extremely suggestive in connection with our conceptions of the nature of the evolution process, for the process more or less clearly resolves itself into three problems, namely: (1) how do these character units arise; (2) how are these character units coordinated into harmonious action within the organism; (3) why do these character units independently evolve, progress or retrogress?

Since the word "character" is very vague, and since the term "unit character" has a special and limited meaning in Mendelian heredity, I propose the new term "biocharacter" as a general designation of the character unit in the organism.

1. KINDS OF BIOCHARACTERS OBSERVED IN PALEONTOLOGY

Biocharacters are those characters, large and small, which through the evidence afforded by ontogeny (*i. e.*, zoology, embryology). phylogeny (*i. e.*, paleontology), or heredity (Mendelian inheritance) are found to be separable from or independent of each other as units in the processes of heredity, of evolution, and of individual development.

Paleontology gives evidence no less positive than embryology and experimental heredity that each organism is made up of a number of such separable characters, including those known as the "unit characters" of Mendelian heredity. In paleontology this separability in heredity and evolution is found to apply to structural, or anatomical, characters of three principal kinds, namely, I, II, III:

I. *New orthogenetic characters (rectigradations of Osborn), characters having an adaptive evolutionary direction from the start, completely separable in heredity, so far as observed continuous in origin.*	II. *New numerical characters, presence and absence characters, meristic characters, characters either adaptive or non-adaptive, completely separable in heredity, so far as observed discontinuous in origin.*	III. *New proportional characters (allometrons of Osborn), quantitative and intensive characters. Generally separable, sometimes blending in heredity, so far as observed continuous in origin, adaptive or neutral.*
Examples	*Examples*	*Examples*
New horns, germinal separability. New dental cusps, germinal separability. Certain new dental folds and plications, germinal separability. Such rectigradations are relatively rare in titanotheres, and much more prevalent in the teeth of horses.	Such characters are of three kinds: (1) Abnormal, or teratological, *i. e.*, reduplication of parts already present, supernumerary digits, teeth, vertebræ, ribs, etc.; (2) normal, by added vertebræ, added ribs, added teeth, added phalanges; (3) minute saltations of structure, minor variations of Darwin. Evolution through the multiplication or diminution of existing parts.	Variations in size and proportions of existing units of structure, *i. c.*, evolution of skull and limb proportions, dolichocephaly, brachycephaly, platycephaly, dolichodactyly, brachydactyly; in brief, adaptive changes of proportion and intensity in existing characters and groups of characters.

The property of separability, divisibility, independence of these biocharacters stands in contrast to that of

their interdependence and coordination of function in the organism. As remarked above the discovery of this property of separability of characters was gradually prepared for in various branches of biology. First, in comparative anatomy, through the observations of the laws of independent degeneration, balance and progression of adjoining parts; second, through embryogeny and ontogeny, in the observation of the hastened or retarded development of adjoining parts; third, through heredity, as, for example, in the mosaic inheritance of Galton; fourth, through paleontology and phylogeny, in the law of acceleration and retardation of Hyatt and, through the observations of many other paleontologists, concluding with the detailed observations of Osborn and Gregory. But the property of separability first shone out clearly and most brilliantly in the discoveries of Mendel in heredity.

This property of structural separability and independence of evolution is closely connected with *separability or independence of function.* Thus separability may distinguish a great number of cells and tissues which are united by a single function, or separability may distinguish a single cell, as, for example, the single giant cells of the spinal cord of certain fishes and amphibians.

It is important, therefore, to realize that this property of separability, the separability of biocharacters, is now observable under six classes of evidence.

2. MODES OF SEPARABILITY OF BIOCHARACTERS IN HEREDITY, IN GENESIS, IN RATE OF EVOLUTION

I. *Biocharacter heritage separability,* unit or exclusive inheritance.

1. *Evidence of Galton, etc., as to mosaic inheritance.*
2. *Character heritage separability,* under the separate "determiner" or "factor" hypothesis of Mendel, Bateson, Morgan, etc., according to which each "biocharacter" of the soma may spring from one or more "determiners" or

"groups of determiners" in the germ. Many characters are not entirely separable, but tend to go in groups or to blend.

3. *Character heritage blending*, Castle, Osborn, etc., as observed in color, size, proportion, etc.

II. *Separability in genesis, biocharacter origin and evolution.*

1. *Origin through saltation*, large heritable leaps (*saltus*, a leap, syn. discontinuity, Bateson).
2. *Origin through minute heritable gradations* (*gradus*, a step), Darwin (minute heritable variations), De Vries (mutations).
3. *Origin through continuity* (*continuatio*, an unbroken series; continuous describes that which is absolutely without pause or break, in contrast to saltation = leaps, gradation = steps). It has been shown (Osborn) that characters continuous in origin may be separable or discontinuous in heredity, as in both the new rectigradations and new proportion characters of the hybrid of the horse and the ass.
4. *Continuous rectigradations*, Osborn, *i. e.*, orthogenetic or adaptive origin followed by adaptive evolution in a single direction, a principle probably corresponding with the *Mutationsrichtung* of Neumayr.
5. *Homomorphic* (Fürbringer) *rectigradations, through independent origins*, the independent production of similar biocharacters in organisms of similar ancestral affinity, the "potential homology" of Osborn, as distinguished from the true homogeny, homology *sensu strictu*, of direct descent of characters from each other.
6. *Mutations of Waagen*, subspecific steps or gradations in evolution of characters under the *mutationsrichtung*, or trend of development in certain directions, of subspecific value, *i. e.*, intergradations between species.

III. *Separability in velocity, biocharacter motion* (*i. e.*, rate of transformation, or relative displacement).

1. *Ontogenetic acceleration,* hastening of development in ontogeny, resulting in certain biocharacters appearing at earlier and earlier stages of development. Hyatt's law.
2. *Ontogenetic retardation,* slowing down of biocharacters in ontogeny, resulting in certain biocharacters appearing at later and later stages of development. Hyatt's law.

1 and 2 result in heterochrony (Gegenbaur).

3. *Ontogenetic balance,* resulting in biocharacters appearing at the same stage as in the ancestral forms.
4. *Phylogenetic acceleration,* appearance of certain homomorphic biocharacters at earlier geologic periods in some phyla than in others.
5. *Phylogenetic retardation,* whereby some homomorphic biocharacters appear at later geologic periods in some phyla than others.
6. *Phylogenetic balance.*

IV. *Separability in grouping, biocharacter cooperation and coordination.*

A biocharacter may be closely linked with others in group function or it may detach itself and connect with another group, *e. g.*, color and speed biocharacters in horses.

1. *Biocharacter correlation into similar adaptive character groups.*
2. *Biocharacter grouping through heredity,* attributable to prolonged ancestral grouping.
3. *Biocharacter compensation,* the gain of one character at the expense of another. The law of compensation of St. Hilaire.
4. *Biocharacter sex linkage,* the union of groups of characters with the function of sex as family or secondary sexual characters.

V. *Separability in proportion characters. Character proportion genesis, separability in the evolution and heritage of proportions.*

1. *Proportion genesis through fluctuation*, theoretically through continued selection of plus and minus variations in a given direction in cases where such variations of proportion are of adaptive significance, as in fluctuating variations of length of neck in the giraffe.
2. *Character proportion continuity in zoologic and paleontologic series*, the unbroken succession in the evolution of changes of proportion, as in brachycephaly and dolichocephaly in kindred races of man.
3. *Character proportion saltation, in hybrids only*, sudden appearance of profound changes of proportion, as in the Mouchamp breed of sheep.
4. *Character proportion heritage separability.* Complete separation in heredity of certain proportional characters, as in crosses between dolichocephalic and brachycephalic types in man.
5. *Character proportion heritage blending*, the origin of blended, or intermediate, forms of certain proportional characters in man and horses.
6. *Character proportion genesis*, partly at least through interaction of separably specific harmones, chalones, internal messengers of the thyroid and other internal secreting glands.

VI. *Separability in orthogenesis, rectigradations, genesis of new adaptive characters from infinitesimal beginnings.*

1. *Independent genesis of similar adaptive characters* in different phyla giving rise to homomorphy.

2. *Evidence of hereditary predetermination or potentiality* of the genesis of similar new characters in phyla derived from similar ancestors.
3. *Continuous evolution of rectigradations in one direction, Mutationsrichtung.*
4. *Mutations of Waagen,* subspecific gradations of character.
5. *Complete separability of rectigradations in heredity,* as shown in the teeth of the hybrids of the horse and ass, all rectigradations being either present or absent but not blended.

3. SUMMARY

It appears from the foregoing classes of evidence that biocharacters are separable in origin, development, evolution, and heredity. First, biocharacters are separable through their many different modes of origin from the germ, either saltatory, gradational, or continuous. Second, biocharacters have different rates of motion, or velocity, in individual development (ontogeny), exhibiting acceleration or retardation. Third, biocharacters have different rates of evolution in different phyla (phylogeny), again exhibiting acceleration or retardation (phyla). Fourth, all the biocharacters of an organism cooperate through various modes of grouping in functional correlation, in compensation, in sex linkage. Fifth, in the hard parts of the body while the biocharacters of form and proportion may originate through continuity, through saltation, or through minute gradations, all the known evolution of proportion biocharacters is continuous. Sixth, in the hard parts the biocharacters of rectigradations have only been observed to originate and develop through continuity.

EVIDENCE OF MULTIPLE FACTORS IN MICE AND RATS

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THE object of this paper is to record certain data on the inheritance of two complex characters and analyze these data together with those obtained in certain analogous experiments by other investigators. This is done with a view to ascertaining what they contribute to our knowledge of the relative merits of the two more or less contradictory hypotheses, multiple segregating factors or a single fluctuating factor which are being advocated by geneticists to explain certain cases of inheritance.

It will be useful at the outset to state in a somewhat definite manner how the alternative hypotheses differ from one another. MacDowell (1916) in a recent paper has clearly and precisely defined the two views. From this start the following statement may be made. The first view supposes that variations of the germ plasm are in the nature of fluctuations. The germ plasm is in a continuous state of variation. The hereditary characters all vary under observation and this is taken to mean that the factors in the germ plasm determining them also vary. Fluctuation in the character is measured and used as a means of detecting and recording a similar though not identical variation in the germinal factor underlying and determining the character under observation. To use a concrete example. In a given species the individuals are of various sizes, some larger, some smaller. Some of this variation is considered as in part due to non-heritable environmental influences. There is, however, a distinct "inheritance" of size. This is considered the result of a variable germinal factor which as Castle suggests may be "Perhaps some substance or ferment which varies in

amount, larger amounts producing larger results'' (1916, p. 55). In crosses between animals possessing distinctly different degrees of the size ''factor'' blending rather than alternative inheritance is supposed to result. This also holds in the case of crosses between races differing only slightly in the degree of the size character. In such cases says Castle ''we do not find it easy to detect segregation'' (1916, p. 55).[1]

The ''second'' view supposes that complex physiological results such as the ''size'' of a certain animal depend upon a complex of genetic factors. All these factors are concerned in the growth and therefore the size of the animal. The germinal determiners of these many factors are considered to be units.[2] As such their behavior in inheritance is definite, non-blending, and essentially Mendelian in nature. The blending, fluctuating nature of the character studied is supposed to be due to environmental factors, to the large number of hereditary factors involved, and to absence or incompleteness of dominance.

With this rather incomplete statement we may turn to an examination of the experimental data.

I. THE INHERITANCE OF SPOTTING IN MICE

Experiments on the inheritance of spotting in mice have been carried on by the writer since 1909. As they progressed it became evident that spotting in mice offered remarkable material for the investigation of the inheritance of a variable character. Spotting as a character is easily measurable and classifiable. It is not affected by changes in the external environment. Variations in

[1] It is interesting to note in this connection, the quotation from Castle given by MacDowell, 1916, page 741, describing the inheritance of minute quantitative differences in intensity of yellow pigmentation in guinea-pigs. The later work of Wright, 1915, also has a distinct bearing on this particular case in that it shows four truly allelomorphic grades of intensity of pigmentation in guinea-pigs. Fluctuation occurs about these four variation centers and Castle's ''complete series of intermediates'' between red and cream color in guinea-pigs is broken into three distinct centers of variation with overlapping range.

[2] East, 1912, has made clear the fact that variation within the unit, under certain conditions must of course be supposed to be possible.

degree of spotting are almost certain to be quickly recognized.

In mice a so-called recessive "piebald" spotting has long been known. In addition a distinctly different hereditary type, commonly dominant, was recorded by Miss Durham (1911), and was further investigated by the writer who found it to be entirely independent in inheritance from the "piebald" type. This "dominant" spotting I have called the "black-eyed white" type of spotting. Further in 1914 a third type of spotting known as "blaze," consisting primarily of a white forehead spot, was reported on by the writer. This type has since been found to be independent of the other two in inheritance. The existence of at least three genetically distinct types of spotting in mice is therefore proven. It is not the purpose of this paper to generalize from this fact, *but to attempt to show that various grades of a single type of spotting may be introduced into a cross and may reappear in the F_2 or back-cross generation, not rarely, but in a considerable proportion of the animals obtained.* This is a common breeding test of segregation as compared with contamination and of alternative as compared with blending inheritance.

The races of mice used offer for the most part extremely homogeneous material. The experimental animals come from closely inbred races which have undoubtedly reached a degree of genetic homogeneity which would lead to definite results in breeding, and a clearer opportunity to observe segregation if it existed. The degree of white spotting in the races of piebald mice used is estimated by recording the approximate per cent. of the dorsal surface which is pigmented. This method is subject, as is any system of arbitrary grades, to a certain amount of error. It is, however, reasonably accurate and affords a fair measure of the degree of pigmentation of any individual.

The first experiment to be recorded is a cross involving an English piebald race descended from black-eyed white mice (Little, 1915). These piebalds vary in the extent of dorsal pigmentation from 20 to 96 per cent. It

will be seen from Table I that there are essentially two modes to the variation curve, one of these occurring at 30 to 44 per cent., and the other at from 80 to 92 per cent. Animals from this race were crossed with dilute brown self mice, and with mice from a yellow and from a mixed black-agouti and black race. The F_1 generation consisted of solid colored young. When these F_1 self young were crossed back with animals from the piebald race, a range of variation was obtained which is shown in the second line of Table I.

TABLE I

PERCENTAGE OF DORSAL PIGMENTATION

	0–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40	41–44	45–48	49–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	81–84	85–88	89–92	93–96
Piebald English stock..	—	—	—	—	2	2	11	27	18	26	18	9	14	6	10	4	12	2	7	17	13	16	15	1
Back-cross piebald....	—	—	—	—	—	—	1	1	6	13	6	7	10	2	11	2	6	1	3	5	6	10	10	5

It will be seen that even though the total of the young obtained is less than in the pure piebald race, its range of variability is essentially the same and there are only four animals among those raised from the pure piebald race which fall outside of the range of variation of the back-cross generation. There is, moreover, no evidence of a single mode in the curve of the back-cross generation, but the modal centers of the parent piebald race are each represented by a large number of young in the back-cross generation.

Another cross in which larger numbers have been recorded is one between Japanese spotted mice and two races of self pigmented mice raised at the Bussey Institution. About two years before the self mice were crossed with the Japanese, certain spotted mice of common ancestry with them were separated off as a different stock. The range of variation in the degree of spotting within this last named race may be considered a fair indication of the probable appearance of the self animals used if they had been spotted. It will be seen from

the first row of Table II that, in this race, animals varying from 56 to 96 per cent. were obtained. There is a tendency for a mode to be formed between 80 and 92 per cent. The Japanese race used is one which for several years has been closely inbred and which holds remarkably true to type. Its range of variation is shown in the second row of Table II. It will be seen that the animals possess from 4 to 36 per cent. of the dorsal surface pigmented. A distinct mode is observed between 13 and 16 per cent.

TABLE II

PERCENTAGE OF DORSAL PIGMENTATION

	0–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	33–36	37–40	41–44	45–48	49–52	53–56	57–60	61–64	65–68	69–72	73–76	77–80	81–84	85–88	89–92	93–96
Tame race...	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	6	10	5	13	11	10	19	9
Japanese spotted....	1	8	74	92	65	23	7	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2 spotted...	—	—	—	—	3	2	5	6	4	10	6	5	9	3	11	5	6	4	8	10	8	9	14	18
Back-cross spotted Gen. 1....	—	—	3	2	4	4	14	14	14	16	5	13	8	7	4	1	6	3	—	2	1	2	5	8
Back-cross spotted Gen. 2....	—	1	1	1	—	—	1	1	—	5	4	6	5	1	3	1	—	1	—	1	—	—	3	3

The F_1 generation obtained from crossing the Japanese race with the self dilute brown or brown agouti animals above referred to, consists of solid colored animals and is therefore not to be recorded. The F_2 generation in which 146 piebald animals have been recorded contains mice ranging from 20 to 96 per cent. pigmented. Among the 146 animals recorded, 20 or 13.6 per cent. fall in grades characteristic of the Japanese grandparents. 94 or 63.6 per cent. are of grades found in spotted animals of the other grandparental race, see Table II, line 3. Two other generations of great interest have been tabulated. The first of these, line 4, Table II, is the result of back crossing F_1 generation animals with animals of the Japanese parent race. In this generation there is distinct evidence of segregation. Fifty-five of the 131 animals recorded fall within the limits of the Japanese grand-

parental race and 35 within the variation limits of the other grandparental spotted ancestor, and of these an appreciable number occur at the upper limit of variability, there being no evidence of a tapering of the curve at this point. This fact would appear to be of marked significance. When first generation back-cross animals are bred *inter se*, a second back-cross generation is produced, which, though it includes only 38 animals, has given extremely interesting results, see line 5, Table II. There are five animals which are distinctly Japanese segregates, and of these three show a degree of pigmentation which would make them easily mistaken for even the extreme variants in the Japanese race. At the other end of the curve it is interesting to note that six of the thirteen young reproducing the condition of the non-Japanese grandparental stock, fall into the two extreme upper classes and may be considered as true segregates rather than due to any chance occurrence of an abnormal physiological condition.

A third cross involving spotted mice has been made. This is between animals showing a small white forehead

FIG. 1

spot and a self race. The dilute brown self race used for one parent is the same that has been already recorded above in the second experiment with mice. The range of variation of white spotting in the blaze or forehead spot race used, is recorded in line 1 of Table III. The grades of spotting designated in Fig. 1, numbers 1 to 4, represent the increasing degrees of white spotting. The animals comprising this spotted race are all of them pure

wild mice descended from a few individuals captured at Forest Hills, and at Wenham, Mass. The F_1 generation obtained by crossing the "blaze" and dilute brown self races is all self in character. The F_2 generation shows a distribution of young recorded in line 2, Table III.

TABLE III

GRADE OF SPOTTING

	$-\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4
Pure blaze animals	7	21	4	20	6	5	3	2
F_2 blaze animals	3	15	1	16	10	7	3	1
F_3, F_4, F_5 blaze animals	25	136	24	107	25	55	7	11

It will be seen that the extremes of variability in F_2 are the same as in the pure inbred wild blaze race. F_3, F_4, and F_5 animals raised *without selection* give a result recorded in line 3 of Table II, and again show the same limits of variation. It will be seen, therefore, that the blaze character is segregated from the cross without apparent modification, although the extremely minute degrees of spotting which it includes are those which one might expect would be modified or entirely swamped if contamination between the gametes of the self and blaze races occurred. This cross together with the cross between mutant and wild rats recorded by Castle to be considered later, give conclusive evidence that even a minute quantitative character segregates after crossing and does not afford grounds for the objection raised by Castle that crosses between races differing slightly in size or like fluctuating characters do not readily show segregation.

To sum up the results of spotting inheritance in mice, it may be said that all the crosses made show a reappearance of grandparental conditions in F_2, back-cross, and other advanced hybrid generations. The reappearance of these grandparental types is frequent enough to lead one to conclude that if segregation in a strictly Mendelian sense is not taking place, that at least the outward appearances of such a process are all of them present.

II. THE INHERITANCE OF SPOTTING IN RATS

Castle's work on the inheritance of fluctuations in the hooded coat pattern of rats is well known to all geneticists, and may now be considered in an attempt to examine the bearing of spotting on the inheritance of fluctuating characters in general. The hooded rats with which

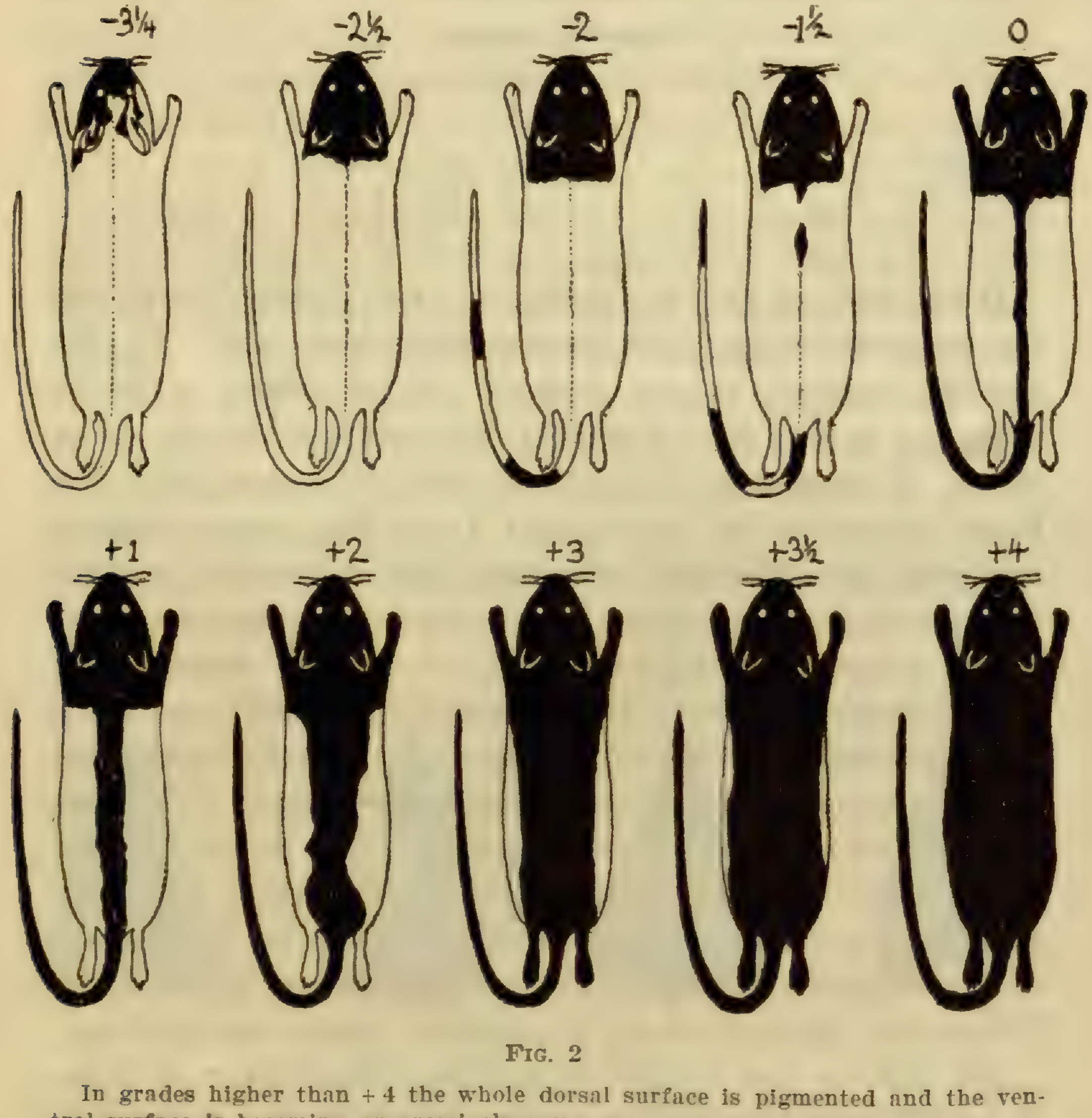

FIG. 2

In grades higher than +4 the whole dorsal surface is pigmented and the ventral surface is becoming progressively more so.

Castle worked have been shown clearly by Doncaster, Mudge, and others, as well as by Castle himself, to be recessive to self or solid colored coat in inheritance. The hooded pattern is, however, subject to wide fluctuation producing a series of rats from those with the whole dorsal surface and most of the ventral surface pigmented,

to rats in which only small spots around the eyes remain pigmented. The following diagram will show roughly the limits of variation and some of the intermediate types as graded by Castle. Throughout the course of his selection experiments, Castle has at times crossed the plus variants with the minus variants or else crossed selected animals from the plus or minus series with wild or with Irish rats. Both the last mentioned varieties are essentially self pigmented animals and are dominant in inheritance to the hooded pattern. The race with which Castle started his selection experiments showed within the first two generations of selection rats varying from grade minus two to grade plus three and three quarters.

Castle and Phillips, 1914, have recorded the results of two crosses between minus variants and plus variants. The first of these crosses was between females of minus two grade in the sixth selection generation and males of plus three and one half or three and three quarters grades in the fifth selection generation. It is possible to use the range of variation among the progeny of animals of these grades within the selection generation from which they are chosen to control the results of the cross. In Table IV[3] the first line represents the range of variation in the progeny of minus two animals of the sixth selection generation. The second line represents the range of variation in the progeny of plus three and one half and three and three quarters animals of the fifth selection generation. It will be seen that there is a space of seven grades between the two limits of variation of the parent races. The cross between these two types produced in the F_1 generation a range of variation shown in the third line of Table IV. It will be seen that for the most part, the young fall in the seven classes between the two parent races. In these seven classes will be found sixty-eight or 73.1 per cent. of the ninety-three young. Two or 2.27 per cent. of the young show grades of pig-

[3] The references to tables and pages appearing in the first column of Table IV, and ensuing tables, refer to Castle and Phillips, 1914.

TABLE IV

Generation	−3	$2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$-\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4
−2 rats, Table 22, p. 41	—	—	3	22	324	368	119	60	51	14	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
$+3\frac{1}{2}$, $+3\frac{3}{4}$ rats, Table 6, p. 34	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	11	5	18	33	67	54	63	30	6
F_1, Table 50, p. 51	—	—	—	—	—	2	—	2	1	8	10	13	25	2	8	11	6	3	1	—	—	—	—	1	—	—	—	—	—
F_2, Table 50, p. 51	—	—	—	—	1	4	9	7	22	24	26	20	49	10	24	24	23	10	20	13	7	5	7	—	—	—	—	—	—

TABLE V

Generation	$3\frac{1}{2}$	$3\frac{1}{4}$	−3	$2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$-\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4	$4\frac{1}{4}$	$4\frac{1}{2}$
$-2\frac{3}{4}$, $-2\frac{7}{8}$ rats, Table 26, p. 41	1	2	1	1	3	5	11	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
$+3\frac{3}{4}$ rats, Tables 10 and 11, p. 36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	9	13	65	124	46	8	1	—
F_1, Table 50, p. 51	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	1	2	4	2	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 50, p. 51	—	—	—	—	—	—	—	—	—	2	—	4	3	8	8	12	9	6	5	3	5	2	2	1	2	1	—	—	—	—	—	—	—	—

mentation characteristic of the plus parent and twenty-three or 24.75 per cent. of the young show grades characteristic of the minus parent race. When the F_1 young are bred *inter se* there is a marked increase in the per cent. of young showing grades of pigmentation characteristic of the grandparents. Ninety-three, or 30.5 per cent., of the 305 F_2 generation young fall in grades characteristic of the minus grandparents. Fifty-two, or 17.0 per cent., fall in grades characteristic of the plus grandparents. The remainder, or 52.5 per cent., fall in the seven intermediate classes.

A second cross made between selected animals is shown in Table V. Females of grade plus three and three quarters of the tenth selection generation were crossed with a male of grade minus three and one quarter of the tenth selection generation. As a control for the females it is possible to use the progeny of grade three and three quarter parents in the tenth and eleventh generations. The reason for using two generations instead of one is in order to increase the number of progeny available, since the tenth generation alone shows such a small number of young that they are not valuable as a significant breeding test. The range of variation in the progeny of rats of this grade is shown in the second line of Table V. As controls for the minus parent, rats of grade minus two and three quarters and two and seven eighths of the tenth selection generation have been used. Although the total of the young produced by them is very small (thirty-one), it is, nevertheless, the only critical data available. The range of variation of these young is shown in the first line of the table. The F_1 generation resulting from the cross of these two diverse selection types is shown in the third line of Table V. The thirteen young comprising this generation all fall in grades between the two parent types. The F_2 generation shows a distinctly greater variability than the F_1 generation and includes four young of grades characteristic of the plus grandparental race. This cross is less conclusive than the previous cross, but

TABLE VI

Generation	$3\frac{1}{4}$	3	$2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$-\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4	$4\frac{1}{4}$	$4\frac{1}{2}$	$4\frac{3}{4}$
$-1\frac{3}{4}$ Parents, Tables 17 and 18, p. 39	—	—	—	—	—	9	8	12	13	33	10	3	2	—	—																		
F_2, Table 42, p. 48	—	—	—	—	—	—	—	2	—	4	6	5	6	13	2	1	3	4	5	3	4	2	1	—	—	—	—	1	—	—	—	—	—
-2 Parents, Table 22, p. 41	—	—	—	3	22	324	368	119	60	51	14	8	—	—	—																		
F_2, Table 42, p. 48	—	—	—	—	—	—	1	1	1	1	2	6	6	7	2	3	6	4	2	4	1	—	—	—	1	—	—	—	—	—	—	—	—
$-2\frac{1}{8}$ Parents, Table 25, p. 42	1	—	4	22	78	251	100	15	1	2																							
F_2, Table 42, p. 48	—	—	—	—	—	1	3	3	1	10	3	9	8	14	—	4	7	8	1	4	3	5	2	4	1	—	—	—	—	—	—	—	—
$+3$ Parents, Table 4, p. 33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	7	12	25	19	35	29	9	4	1	—	—	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5	3	2	3	4	2	1	—	—	—	—	—
$+3\frac{1}{4}$ Parents, Table 6, p. 34; $+3\frac{1}{2}$ Parents, Table 7, p. 35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	9	12	17	47	62	59	59	49	4	1	—	—
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	1	1	1	6	9	8	8	1	—	—	—	—
$+4\frac{1}{4}$ Parents, Table 11, p. 36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	13	5	3	1	1
F_2, Table 42, p. 48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	3	4	2	3	3	—	—	—	—

TABLE VII

Generation	$-2\frac{3}{4}$	$2\frac{1}{2}$	$2\frac{1}{4}$	2	$1\frac{3}{4}$	$1\frac{1}{2}$	$1\frac{1}{4}$	1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	0	$+\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$
$-1\frac{1}{2}\times$ Parents, Table 20, p. 40; Table 19, p. 39	—	1	—	13	23	15	17	16	16	5	3	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	1	5	3	6	12	26	9	9	10	1	3	2	2	1	—	—	—	—	—	—	—	—	—	—	—	—
$-1\frac{7}{8}$ Parents, Table 20, p. 40	—	—	6	31	50	29	12	11	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	—	6	3	4	5	9	14	8	1	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
-2 Parents, Tables 23 and 24, p. 41	7	19	76	843	818	158	26	51	9	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	6	6	12	11	8	3	4	4	6	1	3	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—
$+2\frac{1}{4}$ Parents, Table 2, p. 32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	3	1	1	—	1	—	—	—	—	—	—
F_2, Table 43, p. 48	—	—	—	—	—	—	—	2	4	5	4	16	7	16	22	25	29	24	13	33	14	8	8	6	3	—	—	—	—
$+3$ Parents, Table 4, p. 33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	7	12	25	19	35	29	9	4	1	—	—
F_2, Table 43, p. 48	—	—	—	—	—	—	—	2	—	—	—	1	1	3	3	4	2	2	1	2	1	1	—	—	—	—	—	—	—

does nevertheless show that 5.4 per cent. of the seventy-three F_2 young reproduce the degree of spotting characterizing one of the parental races.

The total number of animals observed, however, is considerably smaller than in the previous experiment and the results are therefore subject to greater error.

We may now consider several crosses made between hooded and wild rats. The F_1 generation in such crosses consists almost entirely of self pigmented animals. It is, therefore, in a comparison of the range of variability observed in the F_2 generation with that observed in the pure hooded race from which the hooded grandparent was taken, that we may expect to find evidence for or against segregation. The first cross to be recorded is between a female of grade $-1\frac{7}{8}$ in the $2\frac{1}{2}$ generation of the minus selection series, and a wild male. As a control for this female, the progeny of $-1\frac{3}{4}$ parents in the second and third selection generations may be taken. It will be seen that their progeny varies between grades -2 and $-\frac{1}{4}$ (see line 1, Table VI), in a total of 90 animals. The F_2 generation shows a range of variability between grades $-1\frac{1}{2}$ and grade $+3\frac{1}{2}$. It is interesting to note. however, that twenty-three or 37 per cent. of the sixty-two animals observed fall within the range of variation of the hooded grandparent. A comparison of grades $-1\frac{1}{2}$ and -2 in Fig. 2 will show how closely F_2 animals approach the extreme condition of the hooded grandparental race.

In the second cross between hooded and wild rats, females of minus two grade of the sixth generation of the minus selection series were used. As controls for these females the progeny of -2 parents in the sixth generation of the minus selection experiment may be used (see line 3, Table VI). The 969 young so obtained fall between the grades $-2\frac{1}{2}$ and $-\frac{1}{2}$. The forty-eight young show a large range of variability from $-1\frac{3}{4}$ to $+2\frac{3}{4}$. Of the forty-eight obtained, twelve, or 25 per cent., fall within the range of variation of the progeny of the hooded grandparental generation.

The third cross was between wild males and females of grade -2 or $2\frac{1}{4}$ in the tenth generation of the minus selection series. As a control for these animals it is possible to use the progeny of $2\frac{1}{8}$ parents in the tenth generation of the minus selection series (see line 5, Table VI). The 474 young so obtained vary between grades $-3\frac{1}{4}$ and -1.

Among the ninety-one F_2 young obtained, eighteen, or approximately 20 per cent., fall within the limits of variability of the hooded grandparents. The range of variability in the F_2 generation is considerable, being from -2 on one side to $+2\frac{3}{4}$ on the other.

Three crosses between animals of the plus selection series and wild rats are recorded. In the first cross (see lines 7 and 8, Table VI) females of grade plus three of the third generation of the plus selection series were used. The breeding capacity of these animals may be fairly judged by considering the progeny obtained from plus three parents in the third generation of the plus selection experiment. The 143 young so obtained range from grade $+\frac{3}{4}$ to $+3\frac{3}{4}$. Only twenty-one F_2 young are recorded, varying between grades $+1\frac{3}{4}$ and $+3\frac{1}{2}$. It is interesting to note that *all of them fall within the range of variation of the grandparental hooded race.*

In the second cross females of grade $+3\frac{1}{4}$, fifth generation and females of grade $+3\frac{1}{2}$, sixth generation, were crossed with wild males. The progeny obtained from animals of similar grade and generation within the selection experiment can serve as controls. The 320 young so obtained vary between grades $+1\frac{1}{2}$ and $+4\frac{1}{4}$. The thirty-eight F_2 young obtained vary between grades $+1\frac{1}{2}$ and $+3\frac{3}{4}$, again, in every case, reduplicating the grandparental forms.

The third cross, which includes only small numbers and is therefore of less relative value than the two previous crosses, was between a female of $+4\frac{1}{4}$, tenth generation and a wild male. The control animals gave twenty-five young ranging from grades $+3\frac{1}{2}$ to $+4\frac{3}{4}$. The F_2 gen-

eration, which consists of only sixteen animals, ranges between the grades $+2$ and $+3\frac{3}{4}$. Six of the sixteen young fall within grades characteristic of the grandparental animals (lines 11 and 12, Table VI).

Crosses between hooded and Irish rats have given comparable results (Table VII). Rats of grades $-1\frac{1}{2}$ generation $3\frac{1}{2}$ minus selection, were crossed with Irish rats. As a control for the hooded animals the progeny of rats of grade $-1\frac{1}{2}$ in the third and fourth selection generation may be used. The total of the progeny so obtained is 112. It will be noticed from Table VII, line 1, that they vary between grades $-2\frac{1}{2}$ and $+\frac{1}{4}$. Only eight or 8.8 per cent. of the F_2 generation vary *outside* of the grandparental grades. The range of variability in this generation is between -2 and $+1\frac{1}{4}$. The second cross is between females of grade $-1\frac{7}{8}$, from the fifth selection generation of the minus series and Irish males (lines 3 and 4, Table VII). As a control for these the progeny of $-1\frac{7}{8}$ parents of a similar generation may be used. The 143 progeny so obtained fall within the grades $-2\frac{1}{4}$ and $-\frac{3}{4}$ inclusive. The fifty-three F_2 young range between grades $-1\frac{3}{4}$ and $+1$. Almost exactly 50 per cent. of them reproduced the grades of the hooded grandparental types.

The third cross is between females of minus two grade in generation $7\frac{1}{2}$ of the minus selection experiment and Irish males. As a control, the progeny obtained from the females of -2 grade in the seventh and eighth selection generations may be used. Such animals produced 2,013 young, ranging from $-2\frac{3}{4}$ to $-\frac{1}{2}$. Sixty-six F_2 young ranged from grades -2 to $+2$. Of these, 75.8 per cent. reproduced the grades characteristic of the hooded grandparental race.

Two experiments are recorded showing crosses of plus selection animals with Irish males. In the first of these, the females used were of grade $+2\frac{1}{4}$ in the second generation of the plus selection experiment. Animals of similar grade and generation may act as controls, although the number of young obtained from them is very small.

The eight young so obtained fall between grades $+1\frac{1}{4}$ and $+2\frac{3}{4}$. From this cross 239 F_2 young have been obtained ranging from grades -1 to $+3\frac{1}{4}$. 129 or 53.9 per cent. show grades characteristic of the hooded grandparent.

The remaining cross was between females of grade $+3$, third selection generation and Irish males. Controls obtained by tabulating the progeny of animals of similar grade and generation show in 143 young, a range between grades $+\frac{3}{4}$ and $+3\frac{3}{4}$. Only twenty-three F_2 young were obtained, ranging between -1 and $+2\frac{1}{2}$. Of these, sixteen, or 69.5 per cent., reproduce the grades characterizing the hooded grandparent.

One other striking cross is recorded by Castle in the case of the rats. This is a cross between "mutant" rats showing a particularly advanced degree of plus pigmentation and pure wild rats. The range of variability of the pure "mutant" race is according to Castle's statement (Castle and Wright, 1916, page 173) between grades $+5\frac{1}{4}$ and $+5\frac{3}{4}$, see Table VIII. The 109 F_2 "mutant" young show a range of variation between the same grades. According to Castle's statement on page 174, "their range of variation does not fall beyond that of the uncrossed mutant race." It would appear as though the evidence of segregation in this case, even to an almost exact degree, was clear. This case, together with that of the type of spotting in mice known as "blaze" (Little, 1914) already discussed, appears to show that races differing from each other in only a minute degree of a quantitative character may show segregation clearly.

TABLE VIII

Generation	+5	$5\frac{1}{4}$	$5\frac{1}{2}$	$5\frac{3}{4}$	$5\frac{7}{8}$
Pure mutants	2	4	28	17	—
F_2 mutant × wild	1	6	49	50	3

To sum up the experiments with rats, it may be said that while it can not be claimed that the evidence is final in regard to the unit nature of the factors involved, they

offer distinct evidence of segregation. That is to say, the combination of gametes formed by hybrid parents reproduce the same zygotic types as did combination of gametes of the pure parent races.

The bearing of the results on the practical breeding of farm animals seems clear. If a complicated and highly variable character as the hooded pattern in rats may be introduced in a cross with a non-hooded form and may be recovered in a large proportion of the F_2 generation, we may encourage crossing as a favorable method of producing new and important breeds. This will be all the more apparent if we agree with the selectionists who hold that the character reappearing in F_2 will be at once amenable to selection and improvement in a desired direction. *Contamination of genes* in breeding experiments which are conducted on a large scale and are followed by rigid selection, need not be considered as a factor of prime importance.

III. Physiological Factors Underlying Growth of Implanted Tissue

The study of the inheritance of spotting in mice and rats has served to give a considerable amount of data more or less directly comparable to that obtained in studies of size inheritance; though presenting, as I shall try to show, certain advantages in their freedom from environmental and age influences and in their definiteness.

We may now consider a very different line of work, which bears a most interesting relation to other studies of the inheritance of complicated morphological and physiological characters. The reaction of various closely inbred strains of mice and their hybrids to implants of a single tumor is definite, and characteristic. It is moreover undoubtedly hereditary as the work of many investigators has shown.

Tyzzer and the writer (1916) have reported results obtained in inoculating Japanese waltzing mice, closely inbred races of common mice and their hybrids, with an

epithelial tumor which originated in the Japanese waltzing race. The parent animals all of them came from races which, because of long continued inbreeding, may be considered to have reached a degree of great genetic homogeneity. It will be seen from the table (loc. cit., p. 403) that all the Japanese waltzing mice inoculated, 58 in number, grew the implanted tumor, while none of the common mice showed continuous growth of the tumor. This absolute difference between the parent races is extremely interesting and offers ideal material for the formation of intermediate conditions of susceptibility in hybrid generations. They further offer a test of the relative value of the hypotheses of multiple factor and of blending inheritance. Sixty-two F_1 hybrids obtained by crossing the Japanese with tame races were inoculated. Of these, sixty-one grew tumors. The tumors grew in most cases fully as rapidly if not more rapidly than in the susceptible Japanese parent races. It is probable that the one animal in this generation which failed to grow the tumor is not a true exception, but that its behavior may be due to poor technique. The second generation hybrids have given an extremely interesting result. Of the 183 inoculated, only three have shown continuous growth of the tumor. This result is surprising in view of the fact that susceptibility appeared to be a dominant character in the F_1 generation. Only thirty-eight animals of the F_3 generation were inoculated; none of them grew the tumor. The striking difference between the F_1 and the F_2 generations suggests at once, alternative rather than blending inheritance. As we have suggested in our previous paper, the most logical interpretation appears to be that a certain physiological condition on which the growth of the tumor depends, is produced in the animals of the Japanese waltzing race. This condition is not found in the tame mice used. The differences between the races are hereditary, since succeeding generations of the Japanese and tame mice behave like their parents. The fact that susceptibility of the tumor occurs in both F_1 and

F_2 generation hybrids shows that the conditions on which the growth of the tumor depends are reproduced in hybrids of the two races. The behavior of the F_1 hybrid generation produced by reciprocal crosses indicates clearly that even when only one of the parents is susceptible and comes from the homogeneous Japanese race, its contribution to its offspring is sufficiently powerful to produce susceptibility in that animal. In other words, we may say that the hereditary factor or factors underlying susceptibility are functional even when present in a "single dose." If there is a single general factor underlying susceptibility we should expect that the F_2 generation would show a large number of susceptible animals. This is not the case. It is possible, however, to consider the behavior of the F_1 generation as being largely due to heterozygosis and not to true inheritance. To eliminate this possibility a back-cross generation was made between F_1 animals and pure Japanese. The sixty-three animals comprising this generation all proved susceptible to the tumor and in a majority of cases grew it as rapidly as did the F_1 hybrids themselves. On the other hand, the F_1 generation crossed with tame mice gave seventy-eight young, all of which were non-susceptible.

In discussing the results, it was further suggested that the explanation which best fitted the facts, indeed the only explanation which fits all of the facts, is that susceptibility depends for its manifestation upon the simultaneous presence of several factors in either the homozygous or the heterozygous condition. The gametes of the Japanese race possess all or nearly all of these in a homozygous condition and therefore produce susceptible animals. The F_1 hybrids possess all of these factors in a "single dose"; they having been contributed by the Japanese parent, and are therefore susceptible. *When, however, the F_1 animals form gametes, these factors, if they are mendelizing and not blending in nature, will be distributed at random in the gametes.* The result will be that the larger the number of factors involved, the rarer will be the inclusion of

all of them within a single gamete formed by the F_1 animals. Since susceptibility in the F_2 generation will depend upon the presence of all of the factors ordinarily found in the Japanese race, it follows that the greater the number of factors involved, the rarer will be the appearance of a susceptible animal in the F_2 generation. It further follows that the susceptible animals of the F_2 generation probably will not possess the factors in a homozygous condition, as did the Japanese grandparents, and therefore they will not, in most cases, breed true, as did the Japanese grandparents, to the character of susceptibility.

For a more detailed discussion of these results from a genetic point of view, the reader is referred to Tyzzer and the writer's earlier paper. It will suffice at the present time to emphasize the fact that it is the inherent nature of the tissue of the host animal that is being studied. The tumor itself is as near a biologic constant as one can obtain. Variation in its growth therefore means variation in the attitude towards it, taken by the host tissue. This attitude appears to be dependent upon a complex of distinct factors. If a change or substitution is made in any one of the members of this complex, a different reaction is obtained. The behavior of the different factors in any such complex is distinctly that of independent units in inheritance. The fact that the reactions of susceptibility and non-susceptibility are dependent on multiple factors seems established. If the tissue of the adult mouse may be analyzed in this way, the conclusion is far reaching. If the reaction of the tissue depends on its substance, and its substance depends in turn on a certain hereditary complex of factors, it is logical to suppose that the rate and extent of development of the tissue as well as other processes of significance to the organism depend, in so far as they are hereditary, on similar complexes of genetic factors. Environment undoubtedly influences certain characters in their development far more than others, and in this respect size appears to be one of the most susceptible.

On the other hand, rate and extent of growth is undoubtedly chiefly dependent on the nature of the tissue involved, and as we have seen there is every reason to believe that this depends on the interactions of a complex of genetic factors which are independent of each other in inheritance.

Other Experiments with Inoculated Tumors in Mice

Loeb and Fleisher (1912) have reported a series of investigations on the hereditary factors underlying the susceptibility of mice to a transplantable carcinoma. As parent stocks they used three races of mice, one American race, and two European. By breeding tests including several generations the percentage of American mice to show continued growth of the inoculated tumor was found to be eighty-four, while those of the European races I and II were twenty-three and three per cent., respectively. The F_1 generation between American and European I gave sixty-eight per cent. susceptible, F_2 from this same cross gave thirty per cent. susceptible. When American mice were crossed with European II an F_1 generation was obtained in which one hundred per cent. of the animals inoculated were susceptible. Only fourteen animals were tested and the number is too small to establish this as an accurate percentage for this generation. The F_2 generation of this cross gave twenty-six per cent. susceptible.

It is interesting to note that in the F_1 generation, where sufficient numbers were obtained to afford critical evidence, the percentage of susceptibility was intermediate between those of the parent races. There is a marked decrease in degree of susceptibility in the F_2 generation. The fact that some of the animals of the American parent race failed to grow the tumors shows that this race is in all probability not homogeneous, and the same is true of the European races since animals within a single race fail to react similarly to pieces of biologically similar tumor. If such is the case, we should expect an intermediate result in the F_1 generation, just as we do when we cross two races differing in size. The F_2 generation should also be

intermediate, though *on the basis of blending inheritance we should expect the percentage of positive animals to be much closer to that observed in the* F_1 *generation than it actually is.*

On an hypothesis of multiple factors underlying susceptibility or immunity to the inoculated tumor the experimental results may well be explained. The F_3 generations of Loeb and Fleisher's work give a result further indicating the possible presence of multiple factors. If a large number of F_2 animals mated *inter se* at random are used to produce the F_3 generation the percentage of susceptible animals in F_2 should be roughly approached in the F_3 generation. 122 animals comprising the F_3 generation show twenty-four per cent. susceptibility as compared with thirty per cent. in the F_2 generation of the same cross. In the F_3 generation of the American times European II cross, sixty-six animals have been inoculated and have given only two per cent. of susceptibility. This difference is possibly due to the fact that F_2 animals forming gametes each closely resembling those of the grandparent European race were unconsciously chosen as parents for this generation.

The interesting part of Loeb's work is the fact that the relative homogeneity of the races of mice which he used approximate closely the conditions in respect to susceptibility and non-susceptibility which one ordinarily is dealing with in size crosses as, for example, Castle's work and also MacDowell's work with rabbits. In none of these cases has there been excessively close inbreeding in either parent race before crosses were made. There is, therefore, no definite complex of factors characterizing the race. As a result the percentage of susceptible animals varies and depends on the character of the particular animals used for breeding. The whole effect produced is to obscure the true nature of the processes involved. In respect to homogeneity the material at the disposal of Tyzzer and the writer possessed a great advantage which became apparent in the definite results produced.

Size inheritance studies have not been recently made with mammals of known ancestry and of approximately pure races. This fact greatly diminishes the value of the results obtained even though they represent work of the most painstaking sort. For this reason the writer started last January a series of experiments on size inheritance in pure races of rabbits. Polish rabbits are being used for the small parent and Flemish giant rabbits for the large parent. It is hoped by a careful study of variation within the pure races to understand more clearly the method of inheritance in the hybrid generations to be produced by later experiments.

To summarize the work with inoculable tumors, one may say that it presents a type of inheritance not explicable on an hypothesis of blending inheritance or of a single variable gene. All the results may, on the other hand, be satisfactorily explained by supposing that the nature of the host tissue and its reaction to the implant depend upon a complex of mendelizing factors.

Conclusion

The fact that three genetically distinct types of spotting exist in mice; that segregation of the degree of spotting occurs in both rats and mice; that segregation of minute quantitative characters like the "blaze" spotting in mice, and the pattern of the "mutant" rats occurs; and finally that the composition and reaction of epithelial tissue in mice depends upon a complex of mendelizing factors, all indicate that in mammals the multiple factor hypothesis is steadily being strengthened as a scientific theory and a practical principle of great interest and importance.

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THE INHERITANCE OF THE WEAK AWN IN CERTAIN AVENA CROSSES[1]

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(In cooperation with the Office of Cereal Investigations, U. S. Department of Agriculture.)

For some time past, the writers have had under observation many different hybrid series of oats. Certain of these offer an excellent opportunity for a study of the inheritance of awns. This paper is a preliminary report aiming to set forth ideas regarding the factor differences between certain types of awns, as a basis for a further study of the relation between awning and other characters of the oat grain.

Kinds of Awns

Practically all of the wild types of oats are characterized by a very strong awn. This awn is typically long, stiff, and geniculate. The basal portion of the strong awn is twisted in a clockwise fashion and either black or dark brown in color. Above the twist, the awn is practically straight until it reaches the knee, at which point it turns sharply and proceeds almost at right angles to its former course and usually in a different plane. The first step in the modification of this type of awn seems to be the loss of geniculation, together with a reduction of the stiffness. Then a further straightening of the awn occurs, leaving it practically straight from the point of attachment to the tip. Such a change is accompanied by a loss of the dark color at the base of the awn. An awn of this last type is usually spoken of as the *weak awn*. The weak awn may vary greatly in length, thickness, and rigidity. In some cases it becomes a mere hair-like appendage,

[1] Paper No. 62, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

FIG. 1. Showing gradations between the spikelet possessing two strong awns, and the awnless spikelet. From left to right are shown: 2 strong awns; 1 strong and 1 weak awn; 1 strong awn; 2 weak awns; 1 weak awn; and the awnless type.

extending scarcely beyond the tip of the lemma and only distinguishable by careful examination. As this awn becomes weaker, it is produced nearer to the tip of the kernel; that is, the rib of the lemma which forms the awn adheres to the lemma for a greater distance before arising as an awn. Among the wild and cultivated types of oats the awns are either characteristically strong, weak, or lacking altogether. In hybrids of these, however, the awns may present all gradations between the awnless and the very strongly awned types. It is usually possible, though, to classify the hybrids as having strong, intermediate, or weak awns. (See Figures I and II.)

METHODS OF STUDY

The parent plants and first-generation hybrids were grown in the greenhouse, and the second and third generation hybrids were grown in the field. In the case of the first-generation hybrids, all of the spikelets on all of the plants could be studied. With the much larger F_2, however, it was found impracticable to attempt to study all of the spikelets on a plant. The study was limited,

FIG. 2. Showing gradations in the weak-awn series, from a spikelet having two weak awns down to the awnless type.

therefore, to one representative panicle from each plant. The spikelets were picked from such a panicle, examined for awns, and placed in a small seed envelope on which was recorded the data as to total number of spikelets, number with one awn, and number with two awns.

MATERIAL

The weak-awned varieties reported on here are the Burt and a strain of the Red Texas. Attention was centered upon the variety Burt. The awnless type in all cases was the cultivated variety Sixty-Day. For the strong-awned oat, a strain of *Avena fatua* was used. All forms had been grown in pure line culture previous to crossing and were uniform within themselves.

Series 514a1 — Burt × Sixty-Day

(Weak Awn × Awnless)

A cross between the Burt and Sixty-Day gave an F_1 which was almost awnless. A few plants had awns on some of the spikelets, but the generation could be considered practically awnless.

The second generation plants showed all degrees of awning, from the perfectly awnless condition to those which were one hundred per cent. awned like the Burt parent. These F_2 individuals were first grouped according to percentage of awned spikelets, with a class range of ten per cent. (Table I.)

TABLE I

F_2 OF 514A1[2]

Per Cent. of Awning	f
0	110
1–10	29
11–20	23
21–30	25
31–40	18
41–50	18
51–60	14
61–70	8
71–80	12
81–90	5
91–99	12
100	66

The occurrence of plants having varying percentages of awned spikelets and forming a more or less continuous series between the parental types, at first suggested a multiple factor condition for awning. A study of the frequencies, however, showed that such an assumption was incorrect. The frequencies of the zero and 100 per cent. classes were too high to accord with an hypothesis of this sort.

When the F_2 plants were grouped in the classes—awnless, partially awned, and fully awned, it was seen that the data approached, in a general way, a ratio of 1:2:1. (See Table II.) The ratio of the first two named classes to the third was found to be 4.15:1, or, on a basis of four, 3.22:.78.

It remained for a study of the F_3 material, however, to throw light upon the number of factors concerned in this cross, and the relation of these factors to each other.

[2] It was necessary to reduce the class range in the case of the 91–99 class, in order to provide the 100 per cent. class. This, however, could have no effect on the conclusions drawn from this table.

TABLE II

F_2 OF 514A1

Awnless	110	274
Partially awned	164	
Fully awned	66	66
	340	340

Behavior of the Fully-awned F_2 Plants.—Seed of three of the fully-awned F_2 plants was sown in pedigree culture for an F_3. The results obtained are shown in Table III.

TABLE III

F_3 FROM FULLY AWNED F_2 PLANTS—514A1

Pedigree	% Awns in F_2	Awnless	Partially Awned	Fully Awned
514a1–22	100	0	0	47
514a1–88	100	0	0	36
514a1–95	100	0	0	20
Total	...	0	0	103

From this data, it would appear that the fully-awned type is the pure recessive. We have already seen that this type has very little influence on the F_1 hybrid, and that it appears in F_2 in only about 25 per cent. of the individuals. Here we find the fully-awned plants breeding true.

Behavior of the Partially-awned F_2 Plants.—Twelve plants which showed some awning in the second generation were planted in pedigree rows for F_3. The percentage of awning in these plants varied from eleven to eighty-seven. In spite of the wide difference as to percentage of awning, their behavior was strikingly similar. With but one or two exceptions, the ratio of plants not fully awned to those which were fully awned was close to 3:1. A total of all these F_3 plants gave the frequencies 419:118, or a ratio of 3.12:.88. In this case the deviation is 2.4 times as great as the probable error, but this can be accounted for by the somewhat wide deviations occurring in cultures 106 and 194. With the exception of these pedigrees, the deviations from the expectancy are not more than twice the probable error. It is of

interest to note that plants with a high percentage of awned spikelets in F_2 did not tend to give a correspondingly high number of awned plants in F_3. Neither did plants with a low percentage of awns in F_2 tend to produce more of the awnless or partially-awned types in F_3.

TABLE IV

F_3 FROM PARTIALLY AWNED F_2 PLANTS—514a1

Pedigree	Per Cent. Awns in F_2	Awnless	Partially Awned	Fully Awned	Not Fully Awned	Fully Awned	Ratio × 4	D	P.E.	D/P.E.
514a1– 55	77	13	16	11	29	11	2.90 1.10	.10	.185	.54
–100	24	36	17	19	53	19	2.94 1.06	.06	.137	.44
–106	11	31	7	5	38	5	3.54 .46	.54	.178	3.03
–119	20	17	8	11	25	11	2.78 1.22	.22	.195	1.13
–128	87	24	11	12	35	12	2.98 1.02	.02	.171	.12
–138	32	13	15	5	28	5	3.39 .61	.39	.203	1.92
–172	15	26	6	8	32	8	3.20 .80	.20	.185	1.08
–194	14	36	19	11	55	11	3.33 .67	.33	.144	2.29
–200	18	24	12	11	36	11	3.06 .94	.06	.171	.35
–216	30	11	18	8	29	8	3.13 .87	.13	.192	.67
–244	60	13	17	7	30	7	3.24 .76	.24	.192	1.24
–264	81	23	6	10	29	10	2.97 1.03	.03	.187	.16
		267	152	118	419	118	3.12 .88	.12	.05	2.40

Behavior of the Awnless F_2 Plants.—Eleven of the awnless F_2 plants were selected for study in F_3, and seed from them was sown in short pedigree rows. The behavior of these plants is shown in Table V. Five of these awnless plants bred true to the awnless condition, giving a total of 249 awnless plants in F_3. The other six broke up into awnless, partially-awned, and fully-awned plants. In no case did the ratio of these types suggest a 1:2:1 ratio. When we group all of the plants which are not one hundred per cent. awned, however, and compare them

with the fully-awned plants, we find that the separate ratios closely approximate a 3:1 ratio. The ratio for all six plants is 2.97 : 1.03, and its deviation is practically the same as its probable error.

TABLE V

F_3 FROM AWNLESS F_2 PLANTS—514A1

Pedigree	Per Cent. Awns in F_3	Awnless	Partially Awned	Fully Awned	Not Fully Awned	Fully Awned	Ratio × 4	D	P.E.	D/P.E.
514a1– 34	0	48	0	0	48	0				
–126	0	75	0	0	75	0				
–185	0	31	0	0	31	0				
–336	0	32	0	0	32	0				
–232	0	63	0	0	63	0				
Total	..	249	..	..	249	..				
514a1–339	0	34	0	8	34	8	3.24 .76	.24	.180	1.33
– 36	0	52	3	15	55	15	3.14 .86	.14	.140	1.00
–176	0	26	6	11	32	11	2.98 1.02	.02	.178	.11
–221	0	11	4	7	15	7	2.73 1.27	.27	.249	1.08
–247	0	18	7	15	25	15	2.5 1.5	.5	.185	2.70
–291	0	29	3	11	32	11	2.98 1.02	.02	.178	.11
Total	..	170	23	67	193	67	2.97 1.03	.03	.072	.43

It is apparent from these data that the F_2 grouping used here includes in the awnless class certain individuals which are heterozygous for awning, and which really belong with the partially-awned plants. According to Nilsson-Ehle (1914) environmental conditions have an effect upon the production of awns. It is quite possible that the failure of these six plants to produce some awns is due to undetermined environmental factors.

A comparison of the relative numbers of awnless and partially-awned plants in Tables IV and V would seem to indicate that awnless F_2 plants tend to give a higher percentage of awnless plants in F_3 than do the partially-awned plants. This may be explained, however, by the

fact that a high percentage of the awnless plants in the second generation were yellow in color and consequently many of them might well carry a factor which inhibits awn formation. Data will later be presented to show a definite linkage between the awn-inhibiting factor and the factor for yellow color in the Sixty-Day.

Series 2501a1 — Burt × Sixty-Day

A second series of hybrids between Burt and Sixty-Day behaved in a manner similar to series 514a1. The F_1 was nearly awnless in both the direct and reciprocal crosses. The F_2 results are shown in Table VI.

TABLE VI

F_2 OF 2501

Pedigree	Awnless	Partially Awned	Fully Awned	Not Fully Awned	Fully Awned	Ratio ×4	D	P.E.	D/P.E.
2501b1	26	96	43	122	43	2.96 1.04	.04	.0909	.44
2501ar1	17	61	28	78	28	2.94 1.06	.06	.1134	.53
2501ar2	4	32	17	36	17	2.72 1.28	.28	.1604	1.75
2501ar3	1	11	4	12	4	3.00 1.00	.00	.2921	0.00
Total	48	200	92	248	92	2.92 1.08	.08	.0634	1.26

2501b1 = Burt X Sixty-Day.
2501ar1 = Sixty-Day X Burt—selection of partially awned F_1 plants.
2501ar2 = Sixty-Day X Burt—selection of awnless F_1 plants.
2501ar3 = Sixty-Day X Burt—unselected F_1.

It will be seen from Table VI that the partially-awned and awnless types of F_1 gave practically the same behavior, each throwing about 25 per cent. of fully-awned plants in F_2.

Series 2401a1 — Red Texas × Sixty-Day

A cross between Red Texas (weakly awned) and Sixty-Day gave an F_1 showing only 1.3 per cent. of awning. The second generation of this cross has not yet been grown.

Discussion of Results in Weak-awn × Awniess.—From the data presented above, the following conclusions may be drawn as to the inheritance of awns in crosses between the weak-awned and the awnless types of oats.

The awnless type is almost completely dominant in the first generation, only a few of the plants possessing awns and those in small percentages.

The second generation gives awnless, partially-awned and fully-awned plants in a ratio which approximates 1:2:1. The totals of data from second generation plants of series 2,501 and 514 are reasonably close to this ratio:

	Awnless	Partially Awned	Fully Awned
Series 2501	110	164	66
Series 514	48	200	92
Total	158	364	158
Expectancy	170	340	170

The behavior of the fully-awned plants shows that this type is the pure recessive, for it breeds true in all cases from the second generation.

All of the partially-awned F_2 plants proved to be heterozygous, throwing in the third generation approximately three plants not fully awned to one fully-awned plant.

The awnless plants of the second generation were found to comprise both homozygous plants of the parental type and heterozygous intermediates which later broke up in the same manner as the partially-awned F_2 plants. It might be expected that some of the awnless F_2 plants would prove to be heterozygous, since awnless plants are found commonly in the first generation.

From these results, it is apparent that we cannot correctly speak of the awnless oat as the dominant type, nor should we restrict the use of the term intermediates to those plants which are partially awned.

It seems very probable that the difference between the weak-awned and the awnless varieties of oats, at least in the varieties studied, may be accounted for by the assump-

tion of a difference in one pair of genetic factors. It may be that awnlessness is a definite character which is a true allelomorph of the fully-awned condition. Some might prefer, however, to consider awnlessness simply as the absence of awning. In that case we must assume the presence of an inhibitory factor to account for the partial dominance of the awnless Sixty-Day over the weak-awned Burt. The data at hand seem to point to the presence of an inhibitor to awning in the variety Sixty-Day. A preponderance of awnless yellows in F_2 and F_3 suggests a linkage of this inhibitory factor with the factor for yellow color in the Sixty-Day. (See Table VII.) Such a finding would be in agreement with the results of Nilsson-Ehle (1914). A very definite linkage of the inhibitory factor with the factor for yellow color has already been observed in a cross between *A. fatua* and *A. sativa* var. Sixty-Day. This will be brought out in a later publication.

TABLE VII

SHOWING THE DISTRIBUTION OF REDS AND YELLOWS IN SERIES 514, WITH PERCENTAGE OF AWNING AS RELATIVE[3]

	0	5	15	25	35	45	55	65	75	85	95	100
Red.....	49	12	10	12	12	8	10	5	8	5	7	38
Yellow...	50	15	12	12	4	10	4	2	2	0	4	21

Certain other crosses with the Burt show that this variety contains a factor for yellow which does not inhibit awning. In the crosses Burt (red) × Swedish Select (white), and Burt × Early Champion (white), the F_2 contained a certain number of yellow-seeded plants, which in turn gave some yellows in F_3. All of these yellows were fully awned. The existence of this yellow factor in the variety Burt has complicated the study of the yellow of the Sixty-Day in these crosses . The fact of the presence of this yellow in the variety Burt should be kept in mind when Table VII is examined.

It will be seen in Table VII, that the red grains are nearly as numerous in the 100 per cent. class as in the

[3] The classes are as follows: –0, 1–10, 11–20, ..., 91–99, –100.

awnless class, and that the other classes are represented in practically equal numbers. In the case of the yellows, however, there are about two and one half times as many in the awnless class as in the fully-awned class. Fifty-seven per cent. of the yellows have less than 20 per cent. of awning, and seventy-three per cent. have less than 30 per cent. of awning. Many of the yellows in the 100 per cent. class are doubtless due to the yellow factor contained in the Burt parent. This factor does not inhibit awning.

Strong Awn × Awnless.—The results of crosses between *Avena fatua* and the variety Sixty-Day (*A. sativa*) agree closely with those obtained in the crosses between the weak-awned and awnless types. (See Tables VIII and IX.)

TABLE VIII

F_2 TOTAL OF SERIES 2516

Pedigree	Awnless	Partly Awned	Fully Awned	Not Fully Awned	Fully Awned	Ratio × 4	D	P.E.	D/P.E.
2516	169	377	201	546	201	2.92 1.08	.08	.04	2.00

TABLE IX

F_3 OF HETEROZYGOUS F_2 PLANTS

Pedigree	Awnless	Partly Awned	Fully Awned	Not Fully Awned	Fully Awned	Ratio × 4	D	P.E.	D/P.E.
687a1–15	61	124	67	185	67	2.94 1.06	.06	.07	.86
687a1–5	79	53	41	132	41	3.05 .95	.05	.089	.56
687a1–1	15	55	24	70	24	2.98 1.02	.02	.12	.17

In a similar study on *A. fatua* × *A. sativa* var. Kherson, Surface (1916) obtained results which agree closely with those presented above. The F_1 plants were nearly intermediate, although "The majority of F_1 spikelets show no awn whatever" (p. 265). In the second generation the following types appeared:

Awnless	133
Intermediate	215
Wild (fully awned)	112

At first Surface assumed that the awnless plants were homozygous and should, therefore, breed true. A test of these plants, however, showed that a certain number were heterozygous. Fifteen out of twenty broke up in the third generation. This might be expected from the fact that some of the heterozygous F_1 plants were awnless. The failure of these plants to produce a few awns is attributed by Surface either to an undiscovered factor affecting awning, or to an environmental influence. It seems quite probable that the variety Kherson may carry a factor inhibitory to awning, similar to the factor in the Sixty-Day.[4]

OTHER CHARACTERS OF THE GRAIN

In connection with the above studies on awning, studies were also made on the presence of basal hairs and the type of articulation of the lower kernel of the spikelet. A strong correlation was found to exist between the fully awned condition and the Burt type (similar to that of *A. sterilis*) of articulation, and also between the fully-awned condition and the presence of medium-long basal hairs such as are found on the Burt grains. When the spikelets were all awnless, the union of the lower kernel and its rachilla was generally of the type found in *Avena sativa* and the basal hairs were either short or lacking.

It is interesting to note, in the crosses between the weak-awned and awnless types, that in every case where a panicle had two awns on a spikelet, all of the spikelets on the panicle were awned. The irregular occurrence of these two-awned spikelets, and the wide variability in numbers on a panicle, makes it seem probable that there is no definite factor for the two-awned condition. It seems more likely that the occurrence of such spikelets is due to environmental influences upon the factor for complete awning.

[4] In some localities the names *Kherson* and *Sixty-Day* are used synonymously.

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SHORTER ARTICLES AND DISCUSSION

NOTES ON THE FAUNA OF GREAT SALT LAKE

In the years during which the writer was zoologist at the University of Utah (1908–15) observations were made on the life of the Great Salt Lake, when time could be spared from multitudinous teaching duties. The animals of this brine lake earliest reported, *Artemia fertilis* Verrill, and the larvæ of the small Dipteron, *Ephydra gracilis* Packard, were naturally the first attraction, since they were abundant, commonly known to science, and readily observable to any one looking for them. A second species of *Ephydra, E. hians* Say, was reported by Aldrich in 1912.[1] A *Chironomus* has been reported also, according to Talmage,[2] but no reference to the authority is given and his own statement is confusing, as he says he has "confirmed the presence of . . . the larvæ of one of the Tipulidæ, probably *Chironomus oceanicus* Packard"! He further states that "The larvæ of the tipula may be taken anywhere near shore during the warm months," but the present writer is compelled to state that neither larvæ, pupæ or adults of either a Tipulid, or of a *Chironomus* was ever observed by him in the Lake, nor are any such reported by Aldrich, an authority on Diptera, in his reports of collecting about Great Salt Lake.[1, 3] Other forms than *Ephydra* might well occur in such a portion of the lake as the great Bear River Bay, where the salt content of the water must be much less, owing to a great influx of fresh water from the Bear River, and to the fact that the bay is partially cut off from the main lake by the causeway of the Southern Pacific Railway. Aldrich, however, has certainly been on the Bear River Bay side of the cut-off, as shown by Plate II, Fig. 8.[1]

In tentatively "trying out" to see what might be a profitable line of study several dilutions were made in order to note the

[1] Aldrich, J. M., "The Biology of Some Western Species of the Dipterous Genus *Ephydra*," *Jour. N. Y. Ent. Soc.*, XX, 77–99. In this are photographs indicating the enormous numbers of *Ephydra* in the lake; also first complete description of *E. gracilis*.

[2] Talmage, J. E., 1900, "The Great Salt Lake, Present and Past," 67–68.

[3] Aldrich, J. M., "Collecting Notes from the Great Basin and Adjoining Territory (Dipt., Col.)," *Ent. News*, XXIV, 214–221.

effect, if any, on *Artemia*. The brine varied in density in the lake at Saltair during the years of these observations, according to season and rainfall, and besides the annual fluctuations gradually became less dense on account of a cyclical period of heavier precipitation and consequent rise in level of the lake. In October, 1909, the density was 1.158, and in April, 1915, 1.136. Dilutions were made by addition of distilled water as nearly as was feasible to the following densities: 1.12, 1.10, 1.08, 1.06, 1.04, 1.02, 1.01[4] These dilutions were placed in small aquarium jars, filled only ¼ to ½ full, and covered to prevent entrance of dust, and undue evaporation. It may be said at once that while Schmankewitsch's classic observations on *Artemia* were of course in mind, it was not hoped to repeat them with the small amounts of water used. However, some data obtained, though incomplete, seems worth recording, since the present writer can scarcely hope ever to have further opportunity for pursuing this line of investigation to the extent it deserves. It is sincerely hoped that some one may be able to further investigate the fauna normal to the unusual ecological conditions of brine lakes.

Resistance of the adult *Artemias* to sudden changes in the concentration of the salts, while greater than anticipated, only showed that they could not indefinitely survive too great a change. Plunged into tap water, they appeared "heavy," sinking at once to the bottom, from which with most vigorous swimming movements they were barely able to rise. Exact data as to length of life in these solutions is not sufficient to offer, but in general they survive a change to completely fresh water but a few hours, and they do not survive for long periods in water in which the amount of salts has been reduced to less than half the normal. In stronger concentrations they survive for sufficiently long periods that it seems likely they would live therein for a normal life period if other conditions were favorable. Kellogg[5] had opportunity in the case of *A. franciscana* Kellogg to note differences in size, color and abundance of individuals which had developed in waters of different densities, and it is interesting to note that he found them largest in waters ranging from 1.11 to 1.13 in density. The latter figure is nearly the same as

[4] See article by Daines preceding this, with exact densities of one series. Part of these observations were made in collaboration with Daines.

[5] Kellogg, V. L., "A New *Artemia* and its Life Conditions," *Science*, N. S., XXIV, pp. 594–596.

that for Great Salt Lake water at the beginning of my observations, at which time the lake was in the rising period of its long cycle of rise and fall, which rise continued at least up to 1915. Some few years prior to 1909 the lake had been much lower and the water at nearly the saturation point for NaCl. I believe 1.13 is somewhere near the mean density for Great Salt Lake.

Interesting facts were noted concerning eggs contained in dilutions made in autumn (see annual cycle below). These hatched in a few days or weeks, and they first hatched in the most dilute water, next in the next more concentrated, and so on up the scale of concentration in nearly regular order. The conclusion naturally presents itself that the stimulus to development lies in the reduction in amount of salts present, but later it appeared (this point was not finally cleared up) that it lay rather in a lack of oxygen resulting from insufficient aeration of the water used. Young thus hatched never reached maturity.

Ephydra larvæ are even more abundant in the lake than *Artemias*. They were found to be remarkably resistant to changes in density of water, as well as to other changes in liquid environment. These larvæ will live at least for days in tap water, but whether they could be brought to maturity in this or in very dilute lake water was not determined. The fact that the puparia drift up on shore in great "windrows" has already been noted by Aldrich,[1] and in the *Canadian Entomologist* for 1891 (original article not seen). The countless swarms of imagoes may be seen by bathers resting on the surface of the water or flying up at will, and it was found to be an easy matter to obtain the eggs by imprisoning a number of these in a covered crystallization dish with clean bottom, partly filled with brine, showing, as suspected, that they drop the eggs freely into the water. As this was not done until near the close of my service in Utah, no experiments were made with the eggs, but attempts to hatch the eggs and rear the insects to the imago stage in dilute lake water and in fresh water should be made. As instances of the resistance of these larvæ may be mentioned the following: In more than one case the larvæ were observed to live months in brine which had evaporated to saturation, and beyond to the point of containing a heavy deposit of crystals and of being completely encrusted on top, and in one such case practically all of the water had disappeared. Among the salt crystals in the little remaining water the larvæ were somewhat inactive, but appeared to be in good condition when water to about the normal amount

was restored to the jar. *Artemia* is resistant to concentration, but not to the same degree as *Ephydra* larvæ. Again, in an attempt to kill larvæ without distortion some were placed in Perenyi's fluid and in this were capable of movement after more than twenty-four hours. In Flemming's fluid they live several times as long as *Artemia,* but I have no record of the exact length of time. I am able to verify with certainty Aldrich's belief that these larvæ do not rise to the surface for air.

Most important of the incomplete observations were those indicating the presence of Protozoa as normal inhabitants of Great Salt Lake. So far as I am aware, no Protozoa have been previously reported from brine lakes. Representatives of this group, notably *Amœbæ,* were first seen in the moderate dilutions after some weeks in the laboratory, which proved to be in a sense cultures. In March, 1910, several jars of a series, including one of undiluted lake water, contained an abundance of these forms. The specimens were of two or three varieties or species, by far the most common being very like *Amœba limax.* I should not have hesitated to call it that in a fresh-water culture. A class of some 15 students was well supplied with *Amœbæ* for laboratory work from one of these jars. Occasionally, in making microscopic examinations of the cultures other Protozoa were met with, but never in numbers. In fact only a single specimen at a time was the rule. Specimens of Ciliata were seen, some closely resembling a species of *Uroleptus,* while at least once a species of *Euglena* was definitely noted. *Chlamydomonas* appeared quite regularly and in great quantity in many of the cultures.[4] I believe several species of Protozoa to be present normally in the Great Salt Lake, but not generally very abundant, as many of my efforts to secure them directly from the lake were failures. However, some were certainly obtained directly from the more or less decayed masses of organic débris which collect in enormous quantity in the great stretches of shallow water along the very flat shore, which masses consist mainly of the gelatinous blue-green alga, *Aphanothece packardii.*[4] (This is the alga "of the *Nostoc* group" mentioned by Aldrich.) In this material it was expected there might be found Nematodes, as they are in so many cases adapted to unusual environment, and so commonly present in decaying substance, but none ever came under observation.

Perhaps a statement of the annual cycle of life of *Artemia* and *Ephydra* may be of interest. For the latter it may be said that

larvæ and pupæ are at all times of the year present in the lake, though less abundant in winter. In the winter months there are but few in the open water, but they are common in the débris above mentioned. Dates for first appearance of adults were not secured, though some appear as early as April; they become common by June, and in July and August are so exceedingly numerous as to be a serious nuisance at times about Saltair pavilion, wind conditions being apparently a determining factor in their coming in swarms about the bathhouses. Ordinarily they keep below the level of the floors, on the piles and on the water surface. Whether any eggs survive the winter can not be stated. There is no evidence of the pupæ surviving on shore, where thrown up by the waves. It seems likely that larvæ and pupæ which remain submerged are the principal, if not the sole means of surviving the winter period.

Adult *Artemias*, the females with fully developed egg sacs, are very plentiful throughout the summer and fall into October. In this month the temperature of the water falls from the summer temperatures of between 25° and 30° C. (exact summer maximum unknown to writer) to as low as 15°–18° C. In November with the temperature as low as 6° C. there may still be seen some few adults. At a December temperature of 1° C. and lower (doubtless goes lower at times for short periods) no adults can be found, as a rule, though reported by Talmage. An abundance of eggs can be secured in fall, winter and spring, especially in the débris near shore. Possibly some may settle into the smooth oolithic sand of the open lake bottom, but I have no evidence that such is the case, and the eggs tend rather to float than to sink. Young appear in April and May, abundantly in the latter month. The earliest record secured for young was March 12 (1910), when a number of minute young were taken. The temperature at that time was 9° C. It will be noticed that *Artemia* differs markedly in its long season of activity from its fresh-water cousin, *Branchipus*, which is so soon gone from its evanescent breeding pools. Correlated with this long active period is the continued presence of abundant water and food and an entire absence of enemies. Enemies play no part in keeping down the numbers of *Artemia*, or of *Ephydra* in the larval stage. In the midsummer bathing season both are present in myriads in the open water, but so transparent are they that the average bather, even the native Salt Laker, seldom notes their presence.

The insect fauna of the lake shore presents material for a study in itself, on which nothing has been published save the material on Diptera by Aldrich, already cited. At the University of Utah I left the beginning of a collection of insects taken in or on the waters of the lake, and I recall that a small Corisid was several times seen and some specimens of it taken swimming immersed in the brine near shore. The species appeared to be the same as one common in fresh and slightly salt and sulphur impregnated waters in the Salt Lake valley.

Probably correlated with the abundance of *Ephydra* adults as food, may be mentioned a "plague of spiders" with which the resort (Saltair) was troubled during one bathing season, about 1910. Several cases of persons being bitten by spiders were reported in one of the Salt Lake papers, though I can not vouch for their authenticity. Certain it is that spiders of more than one species were unusually numerous about the pavilion, as I personally observed, and I learned later that the employees went about with brooms every morning before the hour for opening and destroyed as many as possible. The forest of piles and underpinning beneath the structure, however, was an inexhaustible reservoir from which the supply was constantly renewed. After the close of the season, no other remedy having been found, some employees were kept busy for weeks in boats beneath the huge structure collecting and destroying the egg cocoons, and the next season there was no serious trouble. Many bushels were thus collected. The second autumn this task was again taken up, and since that time no further plague of spiders has appeared, but whether autumn cocoon collecting is still kept up I do not know. I have no doubt that the seemingly sudden appearance of the great numbers of spiders was in reality but the time when, owing to the availability of a great food supply and plenty of space for spreading webs, they reached a high point in numbers, the culmination of years of slow increase.

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ON THE FLORA OF GREAT SALT LAKE

VERY little investigation has been made of the plant life of Great Salt Lake, either of a scientific nature or otherwise. So far as the author of this paper knows, but one attempt has been made in the past so scientifically classify the flora of the lake, and

that attempt was interrupted before it had reached a successful conclusion, so that no publication of the work was made.

Brief mention of what literature we have on this subject seems not to be out of place here.

Professor Farlow (1879)[1] published a description of a blue-green alga, *Polycystis packardii.*

Dr. A. Rothpletz (1892)[2] makes mention of the presence of certain genera of blue-green algæ, connecting them with the formation of peculiar ooliths on shore. Dr. Rothpletz did his work as a geologist, from a geological point of view. He made no systematic study of the lake from a botanical standpoint. The genera of algæ he mentions—*Gleothece* and *Gleocystis*—we have been unable to find in the part of the lake studied, and it might be said, too, that the connection between these and the ooliths has not been generally accepted, even by geologists.

H. F. Moore (1899)[3], in reporting on the feasibility of introducing useful marine animals into the waters of the lake, makes mention, briefly, of the presence of diatoms. As diatoms constitute the chief food of the oyster, their presence was of considerable importance in the investigation, and especially since they are found in greatest abundance at the mouths of rivers where the density of the water is more favorable for the development of the oyster.

Talmage (1900)[4] speaks of the presence of at least three species of algæ—not naming them—and, besides these, he calls attention to the presence of diatoms beds off from shore, as well as living diatoms in the lake.

Miss Tilden published in her distribution entitled "American Algæ," several species from Great Salt Lake. This distribution has not been available, therefore, more definite mention of it can not be made. They are as follows:

Aphanothece utahensis, no. 297,
Polycystis packardii, no. 298,
Dichothrix utahensis, no. 288,
Enteromorpha marginata, no. 266,
Enteromorpha tubulosa, no. 262,
Chara contraria, no. 255.

No other proof of the presence of abundant plant life in the

[1] This paper was not available.

[2] Rothpletz, A., *Bot. Centr.*, p. 35.

[3] Moore, H. F., "The Feasibility of Introducing Useful Marine Animals into the Waters of Great Salt Lake."

lake is needed than the presence of a fauna, abundant in individuals, if not in species. And no further demonstration of the presence of this fauna is required than for one to visit the lake and see, with his own eyes, the water literally teeming with animal life.

The presence of plants is not so evident to the casual observer, although, at certain times of the year, clumps of greenish material, which must at least suggest a vegetable growth, are very plentiful. Areas of a green scum on the surface of the water in more or less protected places also give evidence, directly, of the presence of plants.

The original purpose of this paper was to determine, if possible, the effect as to size of cells and rapidity of growth of different densities of Great Salt Lake water on a species of *Chlamydomonas* which is found there. The problem, then, was to have been purely a physiological one. During the course of investigations along this line, however, other interesting things presented themselves, and a deviation was made from the first plan, so that finally observations were extended to include every species of plant found in the part of the lake investigated.

The observations made covered a small portion of the southern end of the lake at what is known as Saltair Beach. This place is easily accessible, and is at such a distance from any stream entering the lake, that the density of the water there is not affected to any degree.

The following plants are found regularly in the water at that place:

A green alga, *Chlamydomonas* sp., which has been examined by Dr. N. L. Gardner. Dr. Gardner believes it to be a new species —he has not yet published a decription—near to *Chlamydomonas glœocystiformis* Dill, and *Chlamydomonas apiocystiformis artari*. It has a rich green color, and occurs, during the warmer weather, on the surface of the water in many more protected places. It is found in less numbers in whatever decaying plant or animal material may be present. The indications are that this is one of the means the plant has of surviving the winter; since such material brought into the laboratory in the very coldest weather has later developed a rich green growth of the alga.

A blue-green alga, determination of which has been made by Professor W. A. Setchell. He says that it certainly is an *Aphanotheca*, and is undoubtedly the same plant as the one named *Polycystis packardii* by Farlow, and probably also the

same as the one distributed by Miss Tilden from the Great Salt Lake and named by her *Aphanothece utahensis.* On the authority of Professor Setchell, we shall designate it *Aphanothece packardii.*

The plant occurs in small masses, irregular in size, floating in the water and piled up by the waves on shore. These masses show a gradation in color from a deep blue-green, to light brown, and some were colorless; this depending, no doubt, on the conditions of the plants in the individual clumps, and not, as has been suggested,[4] on a variety of species in the clumps. Microscopic examination of this material shows the individual plants in the mature condition, and also in various stages of division by fission. Great numbers of the cells are held together by their gelatinous secretions. The individual plants average about two micra in diameter.

Microscopic examination of the lake water reveals at least two species of diatoms. They probably belong to the genera *Navicula* and *Cymbella.* These plants do not occur in sufficient numbers, in the denser water about Saltair, to be seen with the naked eye.

The fact that putrefaction and decay are taking place in the water, especially near to shore, where organic material is abundant, shows conclusively that bacteria are present.

Here it may be well to suggest that at least some of the plants distributed by Miss Tilden as Great Salt Lake plants, in all probability came from the fresher waters at and near the mouths of rivers, or in the bays formed by the rivers at their place of entrance into the lake. As the present observations were confined to the denser waters, even an indication of the plants referred to—with the one exception noted—was not found.

For the physiological work, water was transferred from the lake to the laboratory, in sufficient amount to make a number of series of dilutions in glass aquaria. These series included solutions of different density, varying in specific gravity from 1.0115 to 1.222, a saturated solution.

Masses—large in some series, but small in one—of *Aphanothece packardii* were placed in these solutions, and, in every case, enough *Chlamydomonas* was thus introduced to start a more or less flourishing growth.

From time to time, measurements were made of the *Chlamydomonas* present in the solutions, and during the first few

[4] Talmage, J. E., "The Great Salt Lake—Present and Past," p. 76. Salt Lake City.

months indications pointed very strongly to the fact that a reduction in size followed transplanting into less dense solutions.

A table below shows the results obtained with the first series. No. 1 contains the water as obtained from the lake, analysis of a sample of which (1910)[5] gives the following:

Constituents	Grams per Liter	% of Sample Taken	% of Total Solids
Total solids	242.25	20.887	——
Chlorine	126.35	10.91	52.23
SO_4-radical	16.00	1.38	6.65
Magnesium	5.18	0.45	2.14
Calcium	0.98	0.08	0.39
Sodium	85.10	7.25	34.68
Potassium	8.82	0.76	3.66
Total of constituents	242.45	20.83	99.75
Salinity	213.32	18.39	88.09

Solutions 0 and 00 were allowed to become further concentrated by evaporation in the laboratory. Nos. 2 to 8, inclusive, were diluted with distilled water. The first measurements were made some time after the series was started to allow the plants to become accustomed to the new conditions, only, indeed, after multiplication had begun. Blank spaces in the table indicate that no motile zoospores were present at that time in the solution.

SERIES NO. 1 STARTED OCTOBER 8

Measurements

No. of Solution	Density	Dec. 15	Jan. 13	Feb. 14	Mar. 10	Apr. 16	June 15	Average
00	1.222				11.7×6.7		15×10	13.3×8.3
0	1.1825	13×7					15×10	13.7×7.8
1	1.1580	13×7.25	12× .5	13×6.5	12×5.4	12×6	12× 7	12.2×6.1
2	1.1239	13×5			11×5	11×6	10× 5	11.2×5.2
3	1.1088	12×5	11×5		11.2×4.5	11×6	9× 5	10.8×5.1
4	1.0822	12.5×5		12×6.5	11.25×5.5	10×5.5		11.4×5.6
5	1.0613		11×4.3			10×5.5	10× 5	10.3×4.9
6	1.0400	9×4.5		9×5	9×5.5	10×5		9.25×5
7	1.0190		8.5×5		9×4.5	8.5×4.5		8.7×5
8	1.0115				11×6.5	10×5	10× 6	10.3×5.8

This table seems to show a slight diminution in size as we pass from the more dense to the less dense solutions, with the exception of the last and least dense of the solutions. It must be said that it is very difficult in measuring *Chlamydomonas* zoospores

[5] McFarlane, Wallace, "The Water of the Great Salt Lake." (Read before the summer meeting (1910) of the Am. Chem. Soc. at San Francisco by Professor W. C. Ebaugh.)

to make definite comparisons as to size. The size of the individual cells even in one solution varies so greatly that one can only obtain an average of the size and then very roughly. The measurements recorded in the table, and all others made, represent the average size of the larger cells in the solutions as far as it was possible under the circumstances to measure them.

The results from the other series did not corroborate definitely the results shown for the first series. Therefore, the only conclusion which can be drawn is, that so far as the present work has shown, variations in density of the water of Great Salt Lake cause no corresponding variations in size of *Chlamydomonas* cells.

In every series but one, decided growth of the *Chlamydomonas* began first in the dilutions about No. 5, and appeared then in order up to No. 1, No. 0, and No. 00, and then down from No. 6 to No. 8. Solutions No. 4, No. 3, and No. 2, as a rule, showed a greater abundance of the zoospores, judging from depth of the green color given to the solutions by them.

The indication is, that water somewhat less dense than that normally present in the lake at its present level is most favorable to development of *Chlamydomonas* sp.

Aphanothece packardii does not grow well in the laboratory cultures. It was interesting to note that they lost their blue-green color and died in the weakest solutions first; this condition following regularly up the series to the most dense solutions. This species gave us no further results. Whether this failure was due to the weak solutions being particularly unfavorable to the alga, or whether it merely indicates that this form is difficult to keep under laboratory conditions, is not certain. The latter seems the more likely conclusion.

The diatoms recovered from the dense waters, on being transferred to the weaker solutions in the laboratory, multiply readily and actually thrive, giving large masses of the characteristic brown growth. In every series, after about a month in the laboratory, solutions No. 1 and No. 2 show a very few live forms which soon die. In No. 3 a few persist; but in No. 4, No. 5, No. 6, and No. 7, they appear abundantly and continue to multiply indefinitely. In No. 8 the live plants are again not very numerous. These observations are in complete harmony with the statements[3] that the diatoms are found in great abundance in the shoaler, fresher waters near to the mouths of the rivers emptying into the lake. They are reported to be especially numerous on

the alluvial fans at the mouths of both the Bear and the Jordan rivers.[3]

The results seem to indicate that the diatoms obtained are true Salt Lake forms, but have become adapted to less severe conditions than prevail in the denser waters. That they are not fresh-water forms which have accidentally found their way into the lake, is suggested by the fact that they do not thrive in the least dense of the solutions of any of the series.

In every series, a cloudiness in the solution appeared as a result of bacterial growth, but the order of appearance in every case was from the least dense solutions up to the most dense. This cloudiness soon disappeared, to reappear at irregular intervals. These facts led to an attempt to determine at least the number of species of bacteria which may be found in the part of the lake studied. So far as we can determine, no attention whatever has been given this phase of the question in the past.

Five distinct organisms, which have adapted themselves to conditions there, were isolated in pure cultures. No detailed study was made of them to determine their species, but enough was done to leave no doubt as to their being at least separate varieties, if one may judge from distinct differences in cultural and morphological characteristics.

Water obtained from the lake under the strictest precautions, was at first plated on phosphorescent, or salt agar, which consists of 40 cc. of normal sodium hydroxide and 25 grams sodium chloride, to 1,000 cc. of plain agar. Later samples of the water were plated on gelatins containing different amounts of the normal NaOH, and NaCl. Better results were obtained with the salt agar than with the gelatin. Later, plain agar was used with good results.

The number of bacteria per c.c. varies between 200 and 625, counts having been made from a number of samples taken in the coldest weather—water 33° F.—as well as in the warmer weather.

A very interesting fact developed; that of the five micro-organisms isolated, three are decided chromogens, each producing abundant pigment. Of the five, one is a diplococcus, which appears sometimes in tetrads and singly. It forms large white colonies on the media used. The other four are bacilli. The one producing no pigment, forms delicate white colonies on the solid media. Of the chromogens, one produces a lemon-yellow; a second produces a bright orange; and the third produces a violet pigment.

CONCLUSIONS

1. Variations in density of the water of Great Salt Lake, cause no corresponding variations in the size of *Chlamydomonas* sp. cells.

2. The indication is, that water somewhat less dense than that normally present in the lake, at its present level, is most favorable to the development of *Chlamydomonas* sp.

3. The diatoms present in the lake multiply best in water much less dense than the dense water at Saltair.

4. At least four species of algæ are to be found in the part of the lake investigated.

5. At least five varieties—possibly species—of bacteria have adapted themselves to the severe conditions in the lake.

In conclusion, I wish to heartily thank Professor C. T. Vorhies for the suggestions he has given me in the preparation of this paper.

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NOTES AND LITERATURE

BIOMETRIC STUDIES ON THE SOMATIC AND GENETIC PHYSIOLOGY OF THE SUGAR BEET

THE beet sugar industry, amounting to hundreds of millions of dollars every year, is the direct result of scientific breeding.

A biologist is loath to demur at any statement which attaches economic importance to scientific work of the kind in which he is interested. The statement may be true. Certainly no one can deny that far greater system and standardization of routine has obtained in the beet sugar industry than in many other branches of agriculture. But the trained scientific man who conscientiously works through some thousands of pages of the literature of sugar beet breeding and cultivation must hesitate before regarding it as a triumph of scientific method. He will rather, I think, feel that science has fallen woefully short of its possibilities in dealing with many problems of great theoretical interest and economic significance.

In no field of agricultural work is the failure of scientific and practical men to cooperate less excusable than in that of sugar-beet breeding. In the routine operations of sugar-beet production chemical data of a relatively satisfactory degree of trustworthiness are obtained for great numbers of individuals. It is not unconservative to say that millions of individual weighings, polarizations or analyses of various degrees of completeness have been made. For two decades the biometric formulæ which might have given meaning to some of these masses of data have been available. Yet the problems which might have been solved have remained unelucidated, to the material loss of both biology and industry.

It seems worth while to illustrate the truth of these statements by some of the advances in our knowledge of the genetic and somatic physiology of the sugar beet which have been made possible by the application of the biometric formulæ.

Consider first of all one of the simplest problems—that of the relationship between the weight of the root and the sugar content of its juice. Notwithstanding considerable discussion this

very simple problem was not definitely solved until 1913 when actual correlations[1] were available. Coefficients ranging from —.224 to —.756 for the relationship between weight of root and sugar content of juice in various short series of data were found. The results were published only after failure in a conscientious and systematic effort to obtain from the agricultural experiment stations really adequate series of data for detailed biometric analysis.

Fortunately the conclusions have since been fully confirmed. Pritchard,[2] in dealing with samples of 250 to 400 beets grown at Fairfield, Washington, found constants ranging from —.253 to —.499. Working with larger samples ($n = 3.784$) from Brookings, S. D., he found $r = -.258$, while Harris and Hogensen,[3] who had a sample of nearly 7,000 beets[4] from Utah cultures, found $r = -.288$.

The splendid work of these investigators leaves no doubt that the percentage sugar content decreases, and, as Harris and Gortner indicated on their limited series of data, in a sensibly linear manner, with increase in weight of root.

These studies, based as they are in some of these series, at least, upon closely bred material, fully justify the criticisms of the conclusions of Andrlik, Bartoš and Urban[5] by Harris and Gortner.[6]

Harris and Gortner have also found negative correlations between weight of root and total solids and coefficient of purity. Thus the larger roots have a smaller quantity of total solids, a lower percentage sugar content and a lower coefficient of purity.

[1] Harris, J. Arthur, and R. A. Gortner, "On the Relationship between the Weight of the Sugar Beet and the Composition of its Juice," *Jour. Ind. and Eng. Chem.*, 5, March, 1913.

[2] Pritchard, F. J., "Correlations between Morphological Characters and the Saccharine Content of Sugar Beets," *Amer. Jour. Bot.*, 3: 361–376, 1916.

[3] Harris, F. S., and J. C. Hogensen, "Some Correlations in Sugar Beets," *Genetics*, 1: 334–347, 1916.

[4] Unfortunately the constant is not altogether trustworthy because the largest and the smallest roots were excluded.

[5] Andrlik, K., V. Bartoš and J. Urban, "Über die Variabilität des Gewichtes und des Zuckergehaltes der Zuckerrübenwurzeln, und über die gegenseitigen Beziehungen dieser beiden Merkmale," *Zeitschr. f. Zuckerindustrie in Böhmen*, 36: 193, 1912.

[6] Harris, J. Arthur, and R. A. Gortner, "Further Notes on the Relationship between the Weight of the Sugar Beet and the Composition of its Juice," *Biochem. Bull.*, 2: 524–529, 1913.

That large roots yield an actually larger amount of sugar is to be expected, and Pritchard's coefficient for the correlations between weight of root and total sugar content is high.

Such results are obviously of great practical significance. Laying aside the possible desirability of modifying planting or cultivation in such a manner as to influence root size, the question of the selection of roots for sampling is one of real importance.

Much of the early American work on the sugar beet was devoted to determining where the crop will give a yield per acre and a sugar content and coefficient of purity satisfactory for economic work. Roots were sent by farmers to the Agricultural Experiment Stations, analyzed, and the results published in a great series of bulletins. But since size and percentage of sugar are correlated, and selection for size in the submitting of samples was rarely guarded against, the great mass of figures have little significance as measures of the merit of the cultures from which the samples were drawn.

If physical characters of the root be associated with sugar content or with purity of the juice, which is technically a highly important factor, physical characters may serve as a guide to selection.

Pritchard has devoted great care to the problem of the correlation between a number of the morphological features of the root and leaf and sugar content, and has determined the average percentage of sugar and average sugar content in synthetic types, *i. e.*, those embodying the most desirable of the morphological characteristics. The results are of technical rather than of general biological interest. The conceptions of a synthetic type—a conception that has already been emphasized in a quite different way by Raymond Pearl[7]—is well worth careful consideration by all those who have to do with breeding problems.

Both Pritchard[8] and Harris and Hogensen have extended their studies of the correlation of characters in the root to that of the interrelationship of the characters of the root and those of the fruiting shoots.

[7] Pearl, R., and F. M. Surface, "Selection Index Numbers and their Use in Breeding," AMER. NAT., **43**: 385–400, 1909. Also, R. Pearl, "Further Notes Regarding Selection Index Numbers," AMER. NAT., **46**: 302–307, 1912.

[8] Pritchard, F. J., "Some Recent Investigations in Sugar Beet Breeding," *Bot. Gaz.*, **62**: 425–465, 1916.

They agree that there is no correlation between the sugar content of a beet and the quantity of seed which is produced, but Harris and Hogensen find a correlation of $.308 \pm .013$ for the relationship between the weight of the seed beet planted and the weight of seed produced, whereas Pritchard, from a number of determinations, concludes that for beets of ordinary size such as are grown for factory use the correlation between root weight, percentage of sugar in roots and quantity of sugar in the seed root on the one hand and the number of grams of seed produced by the seed root is sensibly zero.

Harris and Hogensen find a correlation of about $+.399$ between height of plant and amount of seed produced, about $+.277$ between number of stems and weight of seed produced, and about $+.122$ between number of leaves and weight of seed produced.

Pritchard has shown that there is no correlation between the amount of seed which a beet root yields and the sugar content of its progeny. "The application of this fact to sugar breeding," says Pritchard, "is obvious, as extensive selection may be made for freer seed production without danger of sugar deterioration. Moreover, it affords an opportunity to reverse the order of selection by making the chief elimination in the seed generation and thus greatly reduce the amount of chemical work and increase the effectiveness of the working funds."

The physiological character *time required for maturing seed* has received some attention by Harris and Hogensen, who find a greater height and a higher production of seed in beets requiring a longer period for maturity. The coefficients are, however, low, $r = +.175 \pm .016$ for height and days required for maturing seed and $r = +.195 \pm .016$ for days required for maturity and quantity of seed produced. The correlation between the percentage of sugar in the mother beet and the number of days required for maturing seed is negative, $r = -.129 \pm .014$, *i. e.*, the beets with higher sugar content mature their seed more rapidly.

All these coefficients are very low. The experienced statistician will be cautious in regarding them as significant, remembering that when constants reach minimum values probable errors can not be given their normal weight. Those who have had personal experience in the biological phases of such work will realize its difficulties, and allow the questions of the significance of these correlations to remain open until more extensive

data are available. There are, furthermore, internal evidences of serious heterogeneity in the materials upon which these constants are based. Such irregularities as those seen in the frequency distributions of number of days required for maturing require explanation before coefficients based upon them can be given much weight.

The result of Pritchard's experiments which will arouse the widest interest is the conclusion that with due regard to the probable errors of random sampling, there is no correlation between the weight of the mother roots and the average weight, the average percentage sugar content or the average total sugar content of the progeny roots, that there is no correlation between the percentage of sugar in the mother beets and the average percentage of sugar in their progeny, between the actual amount of sugar in the mother beets and the actual amount of sugar in the progeny roots.

Thus in dealing with our long selected varieties of sugar beets the author is faced to the conclusion:

> Differences in the size and sugar content of individual beet roots show no evidence of inheritance. They are fluctuations, therefore, and apparently play no part in beet improvement.

The practical consequences of such a conclusion should be self evident. One European firm is said to carry out 300,000 analyses annually in the selection of roots for seed production. If the conclusion reached by Pritchard be of final significance, it justifies the assertion that "the cost of analyzing mother beets is an absolute waste of money."

Space precludes a discussion of the data given by Pritchard on the average composition of progeny rows and on the influence of environmental factors in observing genetic differences. From this side his paper must be read, and will later be reviewed in connection with one on the technical features of progeny tests.[9]

His studies show how small are the real genetic differences which may appear, how deeply these differences may be buried under those due to environmental factors, and how difficult in consequence must be the attainment of real progress in the further improvement of so highly selected an agricultural plant as the sugar beet.

[9] Pritchard, F. T., "The Use of Checks and Repeated Plantings in Variety Tests," *Jour. Amer. Soc. Agron.*, **8**: 65–81, 1916.

Pritchard is a mutationist rather than a selectionist.

The selection of choice roots by chemical and physical means has probably played no part in sugar beet improvement except where an occasional root has mutated and thus given rise to a superior physiological species.

One does not need to agree with the form of Pritchard's conclusion to recognize the great value of such studies as those which he has carried out. Full knowledge of the difficulties surrounding a task is one of the essentials to its accomplishment. When all the variables that enter into the problem of sugar-beet production and sugar-beet breeding are known in quantitative terms, it will be possible for the practical man to decide on the basis of the cost of labor and other economic considerations what operations can be dispensed with and what other changes in routine can be profitably made. Operations can then be more properly designated *scientific*.

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THE THEORY OF THE GENE.

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IT is unfortunate that the method of analysis of the problems of Mendelian heredity that has been adopted in one form or another by those who work in this field, has aroused a certain amount of antagonism on the part of those whose work lies in other directions.

In the following pages I shall attempt to explain what the genetic factor means to those who use it, and then try to answer certain specific criticisms of this form of hypothesis, in a hope that a mutual understanding will remove many of the objections that have been made to this method of handling genetic problems.

The objections have taken various forms. It has been said, for instance, that the factorial interpretation is not physiological but only "static," whereas all really scientific explanations are "dynamic." It has been said that since the hypothesis does not deal with known chemical substances, it has no future before it, that it is merely a kind of symbolism. It has been said that it is not a real scientific hypothesis for it merely restates its facts as factors, and then by juggling with numbers pretends that it has explained something. It has been said that the organism is a Whole and that to treat it as made up of little pieces is to miss the entire problem of "Organization." It has been seriously argued that Mendelian phenomena are "unnatural," and that they have nothing to do with

the normal process of heredity in evolution as exhibited by the bones of defunct mammals.[1] It has been said that the hypothesis rests on discontinuous variation of characters, which does not exist. It is objected that the hypothesis assumes that genetic factors are fixed and stable in the same sense that atoms are stable, and that even a slight familiarity with living things shows that no such hard and fast lines exist in the organic world. These and other things have been said about the attempts that the students of Mendel's law have made to work out their problems.

I think, however, that while a few of these charges may appear to be serious, some of them rest on a misunderstanding of what numerical treatment of any problem in science means, and others are due to differences of definition. But the most common misunderstanding arises, I venture to think, from a confusion of the problem concerned with the sorting out of the hereditary materials (the genes) to the eggs and sperms, with the problems concerning the subsequent action of these genes in the development of the embryo.

What genes stand for can be most easily shown by means of a few familiar illustrations. Mendel's cross with yellow and green peas (or any similar case in which two characters are contrasted with each other as a pair) will serve as an example. In the second generation from such a cross the numerical results, viz., three yellow to one green, find their explanation on the assumption that the two original germ plasms (briefly the yellow and the green) or some element or elements in them separate cleanly in the germ cells of the hybrid of the first generation. This cross does not tell us whether the two germ plasms separate as wholes—one from the other—or whether only some part or parts behave in this way.

But the situation changes when two or more pairs of contrasted characters are involved in the same cross.

[1] This objection is not further considered here since it has been dealt with elsewhere ("A Critique of the Theory of Evolution," 1916, p. 84).

For example, when peas that are both yellow and round are crossed to peas that are both green and wrinkled, there appear in the second (F_2) generation not only the original combinations, yellow round and green wrinkled, but also the recombinations yellow wrinkled and green round. Here also the numerical results, 9:3:3:1, can be explained by two assumptions, viz., that, as before, each pair of characters (or their representatives) are separated in the germ cells of the hybrid (F_1) and that each pair "assorts" independently of the other pair. Obviously, here, it can no longer be the wholes of the original germ plasms that separate, for the two pairs of characters behave independently of each other; but there must be separate pairs of elements in the germ plasm that assort independently of one another.

As a matter of fact it has been found that the many pairs of characters that follow Mendel's law are independent of each other in inheritance. The only restriction that this statement calls for is in the case of linked pairs of characters of which I shall speak later.

The germ plasm must, therefore, be made up of independent elements of some kind. It is these elements that we call genetic factors or more briefly genes.

This evidence teaches us nothing further about the nature of the postulated genes, or of their location in the germ plasm. However, even if we postulated nothing more about them than their independence of each other and their distribution in the germ cells, we could still handle the Mendelian results on a purely mathematical basis that would enable us to predict what new combinations should give. This possibility alone would entirely justify the hypothesis as a scientific procedure, whatever carping critics may say to the contrary. In fact Mendel himself did not carry his analysis beyond this point, for he assumed only that definite paired elements that stand in some way for the characters of the finished plant exist in the germ plasm, and that the pairs assort independently

of each other at the time when the members of each pair separate (segregate).

But between the year 1866, when Mendel published his paper, and the present year, 1917—an interval of fifty-one years—much water has run under the Mendelian mill. In consequence we can now add certain further attributes to the rather formal characterization of the gene as deducible from Mendel's law alone. But before I discuss the evidence for these postulated attributes, I must pause for a moment to call attention to a movement that was in certain important respects a forerunner of our present standpoint.

I refer to the views of Roux and of Weismann, both of whom assumed that the germ plasm is made up of particles or determiners, as Bonnet, Spencer, Darwin and others had done before them. Their argument was largely speculative, and not of the same kind as the more recent evidence derived from Mendelian analysis. Moreover in all of Weismann's earlier and best known writings his idea of the units in heredity was more involved than are our present ideas. He thought that whole germ plasms were the units that segregated, germ plasms that differed in one or many determiners, whereas the factorial view that we follow since Mendelism came to the front assumes that the units that segregate are themselves only parts of a whole which is the sum total of all the units. In his latest book, however, Weismann accepted the evidence from Mendelism and modified his ideas accordingly.

We owe to Weismann the popularization of the view that the hereditary material is carried by the chromatin, but especially we owe to Weismann the development of the idea that the sorting out of the hereditary materials takes place at the time of the maturation process in the egg and sperm.

On the other hand, it must be emphatically pointed out that the earlier idea of Roux, adopted by Weismann, that one of the hereditary complexes is sorted out during the

cleavage process of the egg, is no longer acceptable; for there is direct evidence to show that the whole hereditary complex goes to every cell in the body. This conclusion has the most far-reaching consequences for our present views as to how factors produce their effects in the developing organism, for it follows that the machinery that separates the inherited material into its component elements is not the same mechano-chemical process that brings about differentiation in the embryo.

Gene and Character

So far I have spoken of the genetic factor as a unit in the germ plasm whose presence there is inferred from the character itself. Why, it may be asked, is it not simpler to deal with the characters themselves, as in fact Mendel did, rather than introduce an imaginary entity, the gene.

There are several reasons why we need the conception of gene. Let me illustrate by examples:—

1. *The Manifold Effects of Each Gene*

If we take almost any *mutant* race, such as white eyes in *Drosophila*, we find that the white eye is only one of the characteristics that such a mutant race shows. In the present case the solubility of the yellow pigment of the body is also affected; the productivity of the individual also; and the viability is lower than in the wild fly. All of these peculiarities are found whenever the white eye emerges from a cross, and are not separable from the white eye condition. It follows that whatever it is in the germ plasm that produces white eyes, it also produces these other modifications as well, and modifies not only such "superficial" things as color, but also such "fundamental" things as productivity and viability. Many examples of this manifold effect are known to students of heredity.

It is perhaps not going too far to say that any change in the germ plasm may produce many kinds of effects on

the body. Clearly then the character that we choose to follow in any case is only the most conspicuous or (for us) the most convenient modification that is produced. Since, however, these effects always go together and can be explained by the assumption of a single unit difference in the germ plasm, this particular element or gene in the germ plasm is more significant than the character chosen as an index for one only of the effects.

2. *The Variability of the Character is not due to the Corresponding Variability of the Gene*

All characters are variable, but there is at present abundant evidence to show that much of this variability is due to the external conditions that the embryo encounters during its development. Such differences as these are not transmitted in kind—they remain only so long as the environment that produces them remains. By inference the gene itself is stable, although the character varies; yet this point is very difficult to establish. The evidence is becoming stronger nevertheless that the germ plasm is relatively constant, while the character is variable. I shall consider this evidence in another connection. Here I wish merely to register some of the reasons why the idea of the gene is useful.

3. *Characters that are Indistinguishable may be the Product of Different Genes*

We find, in experience, that we can not safely infer from the appearance of the character what gene is producing it. There are at least three white races of fowls produced by different genes. We can synthesize white-eyed flies that are somatically indistinguishable from the ordinary white-eyed race, yet they are the combined product of several known genes. The purple eye color of *Drosophila* is practically indistinguishable from the eye colors maroon and garnet. In a word we are led again to units in the germ plasm in our final analysis rather than to the appearance of a character.

4. *Inference that Each Character is the Product of Many Genes*

We find that any one organ of the body (such as an eye, leg, wing) may appear under many forms in different mutant races as a result of changes of genes in the germ plasm. It is a fair inference, I think, that the normal units—the allelomorphs of the mutant genes—also affect the same part. By way of illustration I may state that we have found about 50 eye-color factors, 15 body-color factors, and at least 10 factors for length of wing in *Drosophila.*

If then, as I have said, it is a fair inference that the units in the wild fly that behave as Mendelian mates to the mutant genes also affect the organ in question, it follows that many and perhaps a very large number of genes are involved in the production of each organ of the body. It might perhaps not be a very great exaggeration to say that every gene in the germ plasm affects every part of the body, or, in other words, that the whole germ plasm is instrumental in producing each and every part of the body.

Such a statement may seem at first hearing to amount almost to an abandonment of the particulate conception of heredity. But in reality it is only a conclusion based on fact. *The essential point here is that even although each of the organs of the body may be largely dependent on the entire germ plasm for its development, yet this germ plasm is made up of independent pairs of units.*

5. *Evidence that Genes have a Real Basis in the Germ Plasm*

In 1906 Bateson, Saunders and Punnett found that certain pairs of characters in sweet peas did not behave independently of each other, but tended to stay together, or to keep apart, in succeeding generations according to the way they entered the cross. Every year more cases of linkage are found, so that there can be little doubt

that this phenomenon is one of the fundamental attributes of Mendelian inheritance

While the linkage relations of genes do not *at present* have any immediate bearing on our conception of the nature of genes, they have a very important bearing on the problem of the localization of genes in the germ plasm.

The original evidence that Weismann accepted to show that determiners are carried in the chromosomes, viz., the evidence based on transmission through the protoplasm-free head of the spermatozoön, was made much stronger from Boveri's evidence derived from experimental embryology.

The argument became still more convincing when the facts of sex-linked inheritance and non-disjunction were established. For, it was found that certain characters have the same distribution as do the sex chromosomes, and secondly by the actual cytological demonstration that the rare exceptions to the rule are due to irregularities in the distribution of the sex chromosomes.

All of this evidence has played a rôle in persuading us that the genes postulated for Mendelian inheritance have a real basis and that they are located in the chromosomes. Finally, in *Drosophila,* where there are four pairs of chromosomes, there are also four great groups of linked genes. This coincidence adds one more link to the chain of evidence convincing a few of us that the gene in Mendelian inheritance has a real existence.

CONSIDERATION OF CRITICISMS

I have tried to make clear how the genetic evidence has necessitated the assumption of genes in heredity, and I have pointed out what seem to me to be some of the attributes that it has been desirable to add to the earlier conception of the gene as our knowledge has increased. Now that the ground is cleared, let me try to answer the objections or criticisms which I mentioned at the start, that have been advanced against this kind of hypothesis.

(*A*) *Assumption of Genetic Factors is Arbitrary*

It has been said that by assuming enough genetic factors you can explain anything. This is true; and it is the greatest danger of the factorial procedure. If, for example, whenever one fails to account for a result he introduces another factor to take care of what he can not explain he is not proving anything except that he is ingenious or only naïve. To make good the introduction of another gene in Mendelian work, its presence must be established by the same kind of evidence as that on which the existence of the original factors was established. For example. Bridges found that after eosin eye color had been crossed to a certain red-eyed stock, there appeared in later generations a new class of eye color (Cream II) that was far lighter than eosin. He isolated this new character and showed that the difference between it and eosin was due to a specific gene that in inheritance behaves like other genes, although its action is not apparent on the normal red eye, but is evident on the eye color eosin. Here, then, through experimental tests, the actual demonstration was made that the change in color of the eosin was due to another gene hidden in the normal stock.

(*B*) *Stability of Genes versus Instability of Characters*

It has been objected that it is unreasonable to assume that genes are relatively stable. This objection is based largely on the fact that characters are notoriously fluctuating, and since characters form the basis of our numerical data from which the idea of the gene is derived, it is supposed that genes too must be variable. This is by all odds the most common criticism that has been brought against the idea of genetic factors and the most difficult one to disprove. There are five answers, however, to this objection.

In the first place it has been shown in a number of instances that the variability of the character is due to a mixed or composite population in which there are sev-

eral genotypes present. In other words, it was because most material is itself not uniform that an exaggerated idea arose concerning the nature of the variability of the character.

In the second place, Johannsen's experiments with Princess beans have shown that when the material is homogeneous in successive generations the variability of the character is due to the environment and is not due to changes in the genotype.

In the third place, any pure stock (and especially one that has been made homozygous by inbreeding), so long as it does not vary, is an argument for the stability of the factorial basis. When changes occur in it as they are pretty certain to do, the fact does not in itself prove that the gene under observation has changed, for other genes that affect the character may have mutated. Jennings has recently said[2] that we maintain the constancy of a given gene by assuming that other genes, rather than the original gene itself, have changed. This would be of course on our part a straight evasion of the issue. The criticism would hold if the question involved were a purely philosophical one, as Jennings might unintentionally lead the reader to believe. Fortunately it is becoming more and more possible to demonstrate that changes of this latter kind do take place; for it is possible with suitable material to show in such cases the exact nature of the change. Wherever it has been possible to do this it has been found that a definite mutation in some gene has taken place, or has been introduced into the culture through crossing.

In the fourth place it has been found that more than one mutant gene may be the mate (allelomorph) of the same normal gene. Since no more than two of them may exist at the same time in a given individual, and since linkage experiments have shown in *Drosophila* that these multiple allelomorphs have the same linkage relations to all other genes (*i. e.*, as we interpret the result, each such

[2] *Jour. Washington Acad. Science*, VII, 1917.

set of allelomorphs has the same locus in the same chromosome) this experimental evidence shows that several allelomorphs of the same gene may exist. An interesting relation in regard to these multiple allelomorphs is that they affect chiefly the same part of the body in the same general way. They *may* give a series of types that is discontinuous, such as the quadruple mouse series: yellow, gray white-belly, gray, black; or the more nearly continuous septuple *Drosophila* series: red, blood, cherry, eosin, buff, tinged, white. Whether such a series of characters is large enough to appear continuous or not is a matter of trivial importance in comparison with the established fact that the genes behind such a series arose in the same way as do other mutant genes, and after they have appeared, are as constant as are other genes. There is no experimental evidence to show that the multiple mutant allelomorphs are more likely to arise from each other than they are from the normal allelomorph, and even if this should be true for individual genes it is no more than is true for other "normal" genes, some of which mutate more readily than others. Emerson has shown for corn that one allelomorph of a series is more likely to mutate than others and we have shown for *Drosophila* that certain normal genes, as the one that mutates to produce vermilion eye color, are more likely to mutate than are others.

When Jennings[3] tries to interpret this evidence of continuous series of allelomorphic characters as breaking down all real distinction between mutation and continuous variation, he leaves out of account certain very fundamental considerations. For example, De Vries himself has always urged that mutations may be very small so far as the character change is concerned; the Svalöf evidence shows this in a very striking way, and Johannsen's beans have been for several years a classic case illustrating how minute the characters depending on

[3] *Loc. cit.*

genetic differences may be. It comes, therefore, somewhat as a surprise when Jennings states:

"Certain serious difficulties appear in this view of the matter; I shall mention merely two of them, for their practical results. One is the very existence of the minutely differing strains, which forms one of the main foundations of the genotype theory. How have these arisen? Not by large steps, not by saltations, for the differences between the strains go down to the very limits of detectability. On the saltation theory, Jordan's view that these things were created separate at the beginning seems the only solution."

It should be remembered too that it is possible to make up just as continuous a series of characters with genes belonging to different allelomorphic pairs (even when they lie in different linked groups) as the continuous series from multiple allelomorphs.

If there were any connections between the gradations of character in allelomorphic series and the order in which the characters appear, such a relation might appear to furnish a support to the view that the assumed fluctuation of factors is a sequential process, and that selection actually helps forward the direction in which mutation is likely to take place, a view that Castle has at times apparently espoused. As a matter of fact, there is no such relation known—the *known* facts are exactly to the contrary; for the actual evidence from multiple allelomorphs shows that genes may mutate in all directions and also that extreme mutations such as white eyes arise suddenly from red and not by graded steps

In the fifth place, the most recent work on *Drosophila* has shown not only that every gene may act (and often does act) as a differential for characters conditioned by other genes, but also that there are genes whose most visible effect is only on certain characters which may therefore be said to be modified by the former. It would be a great mistake to suppose that these modifying genes are unique in any essential respect—the kinds of effects

that they produce grade off into effects that the ordinary genes produce. The chief interest in demonstrating (instead of speculating about) such genes is that they go far towards helping us to a clearer interpretation of certain evidence that was heretofore obscure or misinterpreted. Wherever the history of the origin of these genes is known it has been found to be the same as for other genes and their behavior in Mendelian inheritance is precisely the same. Nevertheless, Jennings has, in the paper already referred to, left certain implications in regard to them that, if not clearly understood, may throw the subject into worse confusion than before. He seems to imply —perhaps he does not really intend to do more—that since through such modifying genes a perfectly continuous series of modifications of a character may exist, all real distinction becomes lost between continuous and discontinuous variation. Now as a matter of fact perfectly continuous characters, if due to overlapping of the separate modifications, can be statistically handled, as Johannsen has done for beans and as Jennings himself has done for size differences in paramecium. Other ways are also known by which the localization in the chromosomes of modifying factors can be studied by methods that no student of Mendelian heredity can afford to reject.[4] All of this is familiar, of course, to Jennings. He means, however, to suggest that if the work on *Drosophila* continues for another fifty years, so many modifiers *may* be found that the characters will form a continuous series. But suppose the mutants do become so numerous that it is impossible to distinguish between any two by inspection. Are we then to reject all the body of evidence that is fast accumulating that the modifying genes are ordinary Mendelian factors? It would be the height of absurdity to throw overboard all this experimentally determined evidence as to the actual method of origin and inheritance of these genes because a time may come when members of a series have become so numerous that we

[4] See "Mechanism of Mendelian Heredity," pp. 192–4.

will be too much bored to make the tests that will distinguish a given new member from some one or other of the old ones. But Jennings may reply, suppose the selectionist claims that his material is already in this finely triturated condition! If, so, the answer is that by suitable selection experiments an analysis may in many situations still be made, and, secondly, the evidence, even from Castle's rats, is far from establishing that he is dealing with such a sublimated process. On the contrary, there is much in them to indicate that they may be capable of being handled by rather simple Mendelian methods, as MacDowell has shown.

As a matter of fact, when indistinguishable characters are the product of one or another *modifier*, the identification of the two genes involved, as independent, is perfectly easy and certain by means of linkage relations. If a particular material is not sufficiently worked out to make this test possible, is that a sufficient reason why we should refuse to accept evidence where it can be obtained? And if there are indistinguishable characters that are the product of one or of another *allelomorph*, of course it can not be determined which allelomorph produces the result; but as, ex hypothesi, each allelomorph produces the same indistinguishable result, a discussion of such a question would be as profitable as the ancient one of the number of angels that can stand on the point of a needle.

In conclusion then it may be said that by stable or constant genes we do not necessarily mean that the gene is absolute in the sense that a molecule is absolute, for we can not know this at present. We might mean by stable genes that even if there is a variability of the gene the variation takes place about a mode; and if in a given individual the extreme of variation was caused by a corresponding extreme in the variation of the gene, still the experimental evidence shows that in the many cell-generations through which that individual's germ cells pass to produce the sperms or the eggs, the genic variation, if there is any, is still about the *original* mode and that no

new mode has been established unless a mutation has occurred. This latter interpretation is indeed in contradiction to the idea that the gene is a single molecule, for molecules are not supposed to vary about a mode. At present either interpretation is compatible with the evidence, which does not discriminate between them.

(C) *Non-Contamination of Genes*

At the time when Darwin wrote and for many years afterward it was supposed that any new or unusual trait of character would become obliterated by repeated crossing with the normal or average individual of the species. This was perhaps the most serious difficulty that Darwin's theory of natural selection met with. It will be recalled that in order to overcome it Darwin made a concession that in principle amounted to an abandonment of the origin of characters through natural selection of chance variations. He admitted that only when a new character appeared in a large number of individuals at the same time was there an opportunity for its perpetuation.

In sharp contrast to this earlier view, all the evidence from Mendelian heredity goes to show that however often a new character, that rests on a genetic change in the germ plasm, may have been kept out of sight by crossing to dominant individuals, whenever the character emerges from the cross, it shows at once that its gene has not been contaminated by contact with other genes. This conclusion is an enormous gain for the theory of natural selection based on chance variation, and at the same time is an equally strong argument to show that genes remain stable, and are not infected or mixed in the presence of other contrasting genes.[5] Let me illustrate by a case of my own.

[5] Those who in their haste try to show that Darwin must have meant by fluctuating variation small mutations, since he assumed such fluctuations to be inherited, might well ponder the difference between the two kinds of variation cited above. If Darwin had realized the difference referred to, he would not have had to make the damaging concession forced upon him by his critic, a professor of engineering, Fleeming Jenkin, in the *North British Review* (June, 1867).

There is a mutant called "notch" (Fig. 1) characterized by a serration at the ends of the wings. The factor that causes this is sex-linked, dominant in regard to the

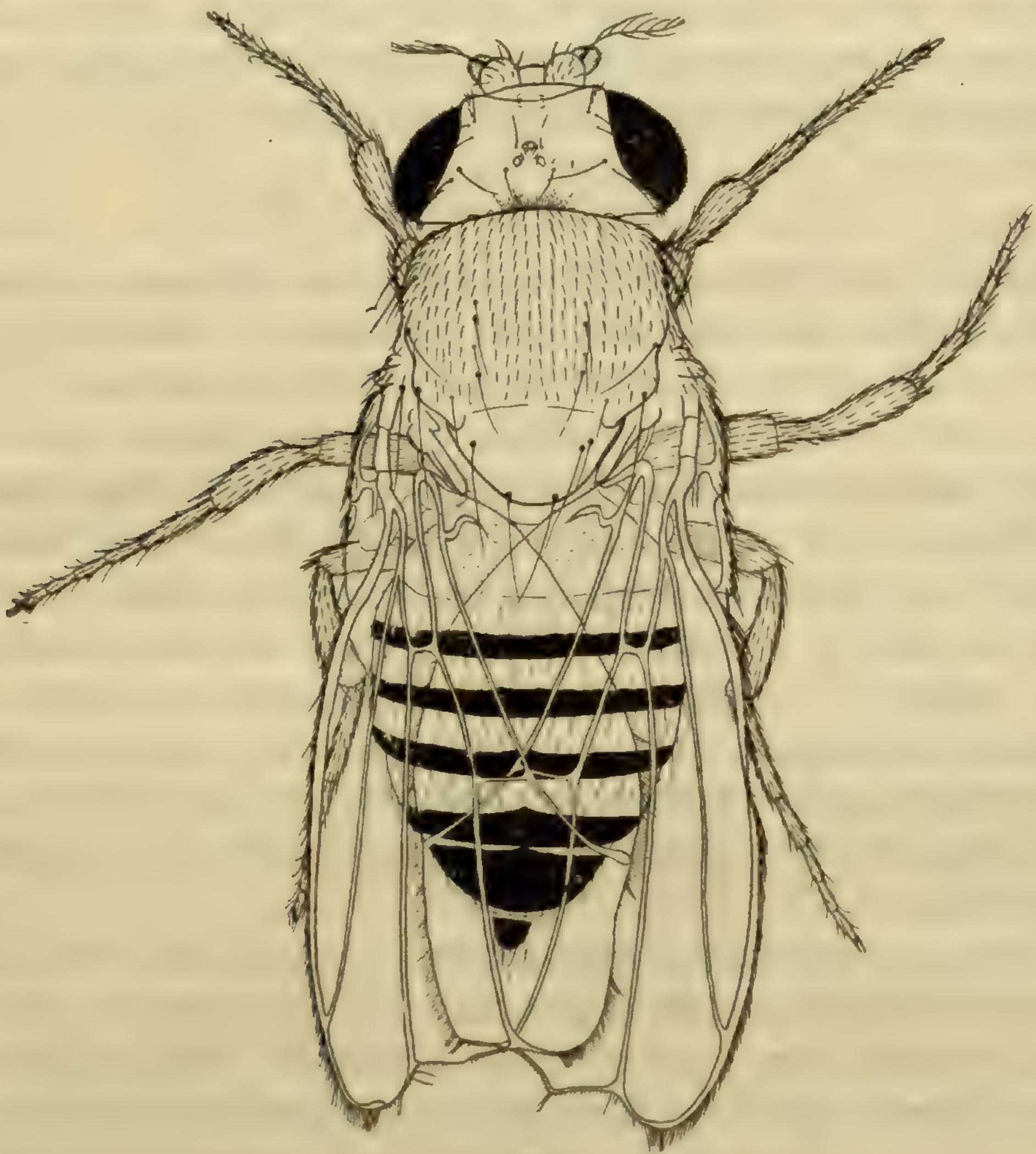

FIG. 1. Notch female.

wing character but recessive in its lethal effect. A female with notch wings carries the gene in one of her X-chromosomes and the normal allelomorph in the other X-chromosome. Half of her sons get the former and die, the other half get the latter X-chromosome and live. As there are no lethal bearing males, the females must in every generation be bred to normal males. For twenty generations such matings have been made. Each time there have come together in the same female one X-chromosome carrying the gene for notch and its mate carrying the normal allelomorph. Selection of those females that

showed the least amount of notch, changed, after a few generations, the outward character of the notch stock so that at least half of those females that carry the notch gene came to have normal wings. It might have seemed that the gene itself had changed, possibly through contamination with the normal gene, were such a thing possible. On the contrary, if these females with normal wings are outcrossed to a male of any other stock, all the daughters that carry the notch gene have the notch in the original (atavistic) condition, showing that the gene still acts in all its original strength. Moreover, suitable experiments have shown that as a result of selection, a modifying gene, already present in the original stock, has been isolated. This gene modifies notch (although it produces no visible effect on the normal wing) in such a way that the notch is less likely to appear. The evidence furnishes the twofold demonstration that the gene for notch has not changed through contamination, and that there is present a new and definite gene that does account for the change.

(*D*) *Methods of Inheritance that are not Mendelian*

It has been claimed that Mendelian inheritance is only one kind of inheritance and applies to only a limited group of characters. It has even been implied that the kind of characters involved in the process of evolution can not be inherited in this way because, it is affirmed, evolutionary characters are not like Mendelian characters. It is known that certain plastids, such as chloroplasts, that lie in the protoplasm are transmitted as a rule only through the egg protoplasm. There can be no doubt that this sort of transmission takes place. In principle it is not different from the transmission of certain kinds of bacterial diseases like that of pebrine in the egg of the silkworm moth. Any inclusion in the cytoplasm capable of increasing there by division would be mechanically carried to all the new cells arising by division and there-

fore into the egg cell also. Should the sperm cell strip itself free of most of that part of the cytoplasm that contained these inclusions, the spermatozoa alone of all the cells in the body would be free from these cytoplasmic materials, and in consequence would not transmit them.

So long as we recognize with what we are dealing here it is largely a matter of personal choice whether we prefer to include plastid transmission through the egg (or even through the cytoplasm of the sperm in special cases) under the term heredity.

The number of cases in which plastid inheritance is known to occur is very limited,[6] while Mendelian heredity includes the vast majority of characters about whose inheritance we know something definite.

But it is a far cry from these cases of transmission of plastids to the view that the cytoplasm transmits equally with the chromosomes; or that the cytoplasm transmits the fundamental attributes of the organism and that the chromosomes transmit only the more superficial characteristics—a view that Boveri discussed in detail in 1903, and which was a favorite topic of his on several later occasions. He changed entirely as the evidence came in and finally abandoned the view in his last paper (1914).

This is an old and familiar topic with embryologists, but since it has been recently revived, a brief statement in regard to it may not be out of place. Fortunately this view is no longer a matter of opinion but of experimentally determined evidence.

In 1912, Toyama described some cases in silkworm moths of what is known as maternal inheritance—cases in which certain characteristics that develop in the hybrid embryo are like those of the maternal stock. He found cases involving the color of the yolk, shape of the egg, and the pigment (not present as such in the egg) that develops after the serosa is formed. By breeding tests it

[6] If chondriosomes are "formative" materials as certain writers claim, the type of plastid inheritance may include a larger group of characters than we suppose at present.

was made clear that the cytoplasm transmits these characteristics only because they have been impressed on the cytoplasm by the chromosomes at some earlier stage in the history of the egg cell. They are strictly Mendelian (Fig. 2).

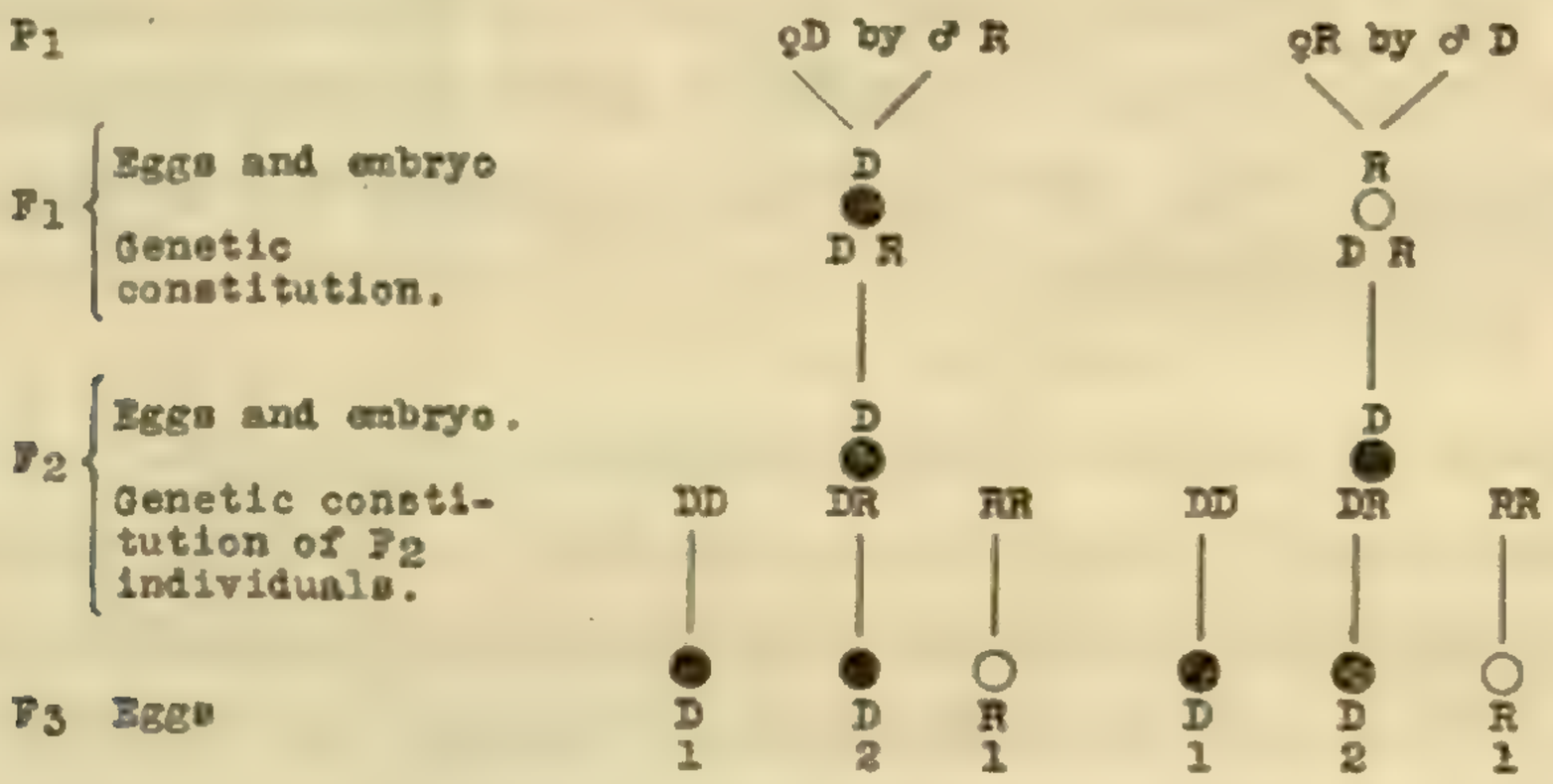

FIG. 2. Maternal inheritance in the silk-worm moth according to Toyama.

It has also been suggested that the chromosomal, Mendelian genes affect only trivial characters such as color, while the more *fundamental* characters are carried in the cytoplasm.[7] There are in reality no grounds for assuming that some characters are more fundamental than others; or that such hypothecated fundamental characters have a different mode of inheritance. The old-fashioned distinction between ordinal, class, family and generic characters has long been recognized as entirely artificial and conventional while the so-called promorphological characteristics such as shape of egg, type of cleavage, axial relations are as variable as are other characteristics, and some of them, such as shape of egg, location of micropyle, etc., have been shown to fall under the Mendelian formula. Take, for example, the following list of characters and try to decide whether they are fundamental (generic) or only trivial; they are all Mendelian in some cases at least:

Sterility, several types of which are recognized as Mendelian;

[7] Boveri, 1903; Loeb, 1916.

Sex, the inheritance of which is shown in many cases to be associated with sex chromosomes;
"Apterous," loss of wings in certain stocks of *Drosophila;*
"Eyeless," partial or complete loss of eyes;
"Extra legs," duplication of part or of entire legs which in one race shows Mendelian sex-linked transmission;
Heliotropism, loss of positive response to light in one stock of *Drosophila.*

Until there is forthcoming some direct evidence that the cytoplasm apart from its contained plastids transmits more fundamental characteristics than the chromosomes, the claim that such a difference exists is not only entirely speculative, but has been shown not to be true for a number of characters. No doubt the idea arose from the fact that when the egg begins to develop it is the protoplasm that exhibits most of the phenomena concerned with the early development of the axial and bilateral relations, the type of cleavage and the formation of the organs of the embryo. But this kind of evidence shows no more than that these characteristics are then present in the cytoplasm; it does not show whether they have come from the chromosomes in the early history of the egg cell, or were, as assumed, inherent properties of the cytoplasm as such. In recent years, however, it has been possible in a few cases, like those of Toyama, to get experimental evidence bearing on this point and it has shown beyond dispute that such cytoplasmic types of behavior are impressed on the cytoplasm by the chromatin in the same way presumably as are all Mendelian characteristics.

No one has denied, so far as I know, that the cytoplasm is essential for development. That it is transmitted largely, if not entirely, through the cytoplasm of the egg is too well recognized to debate, and that it may contain substances that have never been a constituent part of the nucleus and which may form the basis through which material of nuclear origin may act must be freely granted

as an important theoretical possibility. But it must not be forgotten that the only characters that we know anything about in genetics are under nuclear control with the exception of plastids that can themselves multiply in the cytoplasm.

There is a special case of inheritance that has been called cytoplasmic that may equally well have a chromosomal explanation. Goldschmidt finds that he can account for certain of his results in gypsy moths by ascribing certain values to the cytoplasm. Thus he says the two factors for femaleness (FF) are transmitted from the mother to her daughters and the latter transmits again to their daughters, etc. In other words, the factors are carried only in the egg-producing line. Goldschmidt concludes that the evidence proves that the "FF complex is inherited . . . in the protoplasm of the female." Now in moths in which the female is the heterogametic sex, the Y chromosome (or the W chromosome to use a different nomenclature) is transmitted only by the female line and should this chromosome carry the factors in question all the requirements of the experiment would be fulfilled. There is no way of determining from this evidence alone whether the case belongs to the plastid type of inheritance, or is a case of W inheritance, except by finding species in which the female normally lacks the W sex chromosome, or by some anomalous condition has lost it as in the 55 chromosome females that Doncaster has found in the moth *Abraxas*.

There is still another rôle that the cytoplasm may play in determining the nature of the next event to occur. In Phylloxerans it has been shown that a whole sex chromosome is eliminated from the small eggs and in consequence a male results from them. The presumption here is that the effect is through the cytoplasm determining the distribution of the chromosomes, but it must be conceded that the same environmental changes that affected the cytoplasm may have had a simultaneous effect on one of the sex chromosomes. In the case of certain generic

crosses in pigeons, Riddle, confirming Whitman's discovery, finds that when an enforced series of eggs are laid, their chemical composition is changed and that they produce at certain times a preponderance of males. Since the female here is the heterozygotic sex (ZW) the results are such as would follow a direct influence on the sex chromosomes when the polar body is eliminated. Information concerning sex-linked inheritance in these forced offspring should settle the question.

To sum up, it may be said that "plastid" inheritance is at present the only known method of transmission of factors that does not come under Mendel's laws. The three principal kinds of Mendelian inheritance known at present fall into the following groups:

1. Autosomal inheritance, where transmission is equally to both sexes, or to all individuals of hermaphroditic species.
2. Sex-linked inheritance, (*a*) where the distribution of characters coincides with the distribution of the X chromosomes in the *Drosophila* type, and of the Z chromosomes in the *Abraxas* type; and (*b*) where the distribution of characters coincides with the distribution of the Y chromosome (as illustrated by the fertility of the male of *Drosophila* that depends on the presence of the Y chromosome) or of the W chromosome in moths.
3. Inheritance due to unusual distributions of chromosomes, as seen (*a*) in doubling of their number (tetraploidy); (*b*) in non-disjunction, as in the 15-chromosome type of *Œnothera* and the XXY type of female in *Drosophila;* (*c*) in irregularities of synapsis as seen in species hybrids such as *Pygæra.* This group (3) is at present only provisional and will no doubt be broken up at some future time into its different parts.

The case of maternal inheritance, spoken of above (other than Y or W linked or plastid inheritance), has been

shown to be only deferred Mendelian inheritance traceable to the chromatin of the nucleus in which the characters shown by the egg or the embryo have already been determined before fertilization by the chromatin of the mother alone. In consequence the appearance of the Mendelian ratio is deferred to a succeeding generation (F_3).

(E) Action of Genes during Embryonic Development versus their Distribution in Heredity

On several occasions I have urged the importance of keeping apart, *for the present at least,* the questions connected with the distribution of the genes in succeeding generations from questions connected with the physiological action of the genetic factors during development, because the embryological data have too often been confused in premature attempts to interpret the genetic data. It has been urged that such a procedure limits the legitimate field of heredity to a process no more intellectual than that of a game of cards, for Mendelism becomes nothing but shuffling and dealing out new hands to each successive generation. My plea is, I fear, based largely on expediency, which may only too easily be interpreted as narrow-mindedness; yet I hope to be amongst the first to welcome any real contribution concerning the nature of genes based on the chemical changes that take place in the embryo where the products of the genes show their effects. In fact I do not know of any other more direct way in which we can ultimately hope to find out the nature of the materials that we think of as genes in the germ cells.

But experience has shown, I think, that only too often the embryological data have been used to interpret the transmission data to the detriment of both subjects; I regret to see the inevitable difficulties that are natural, at present, to the field of embryology thrust upon the other subject, where the problem is comparatively simple; and

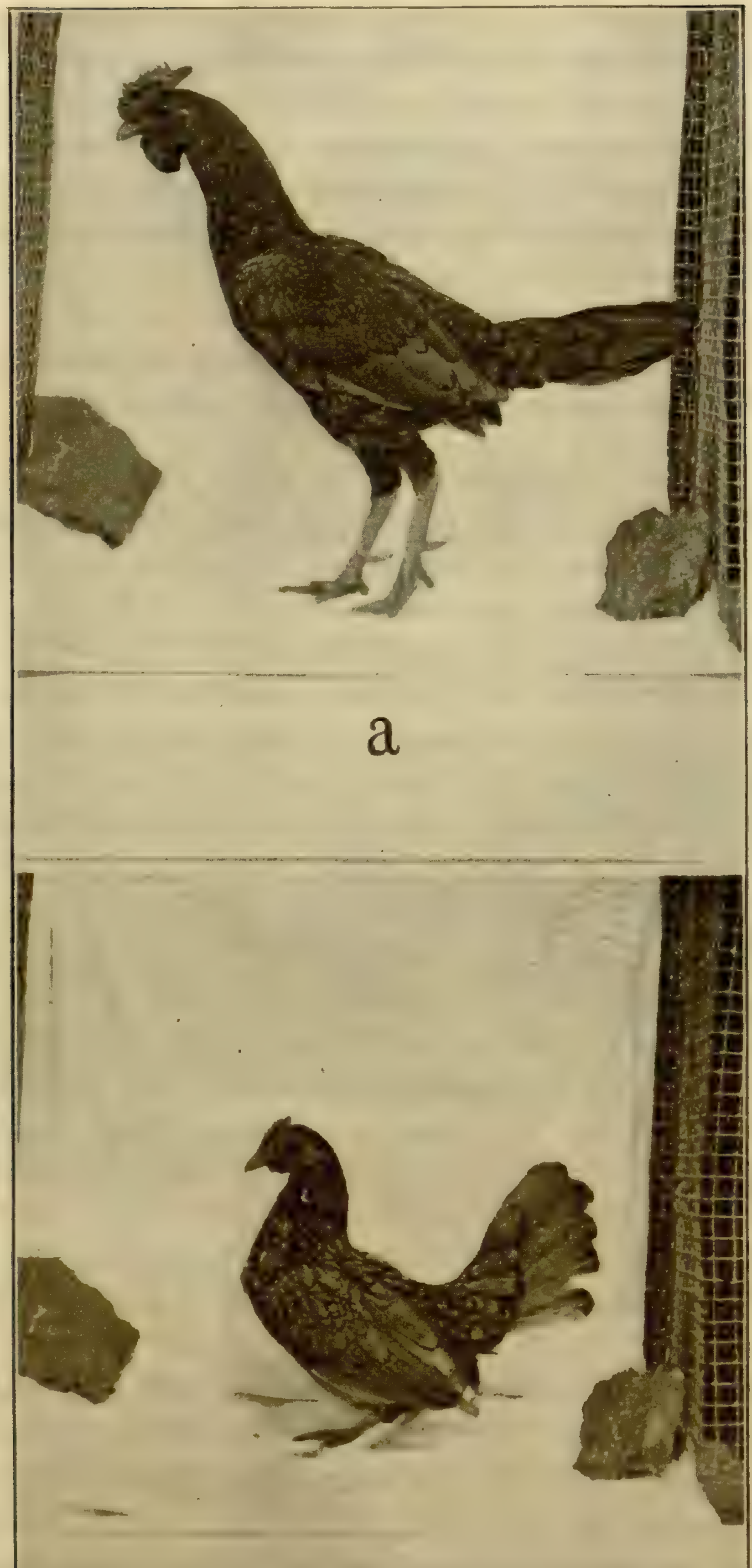

FIG. 3. Male, *a*, and female, *b*, Sebright.

so far as it has progressed, understood. Do not understand me to say that I think all the problems of heredity have been solved, even with the acceptance of the chromosomal mechanism as the agent of transmission.[8] In fact, I think that we are only at the beginning even of this study, for the important work of McClung, Wenrich, Miss Carothers and Robertson shows that there are probably

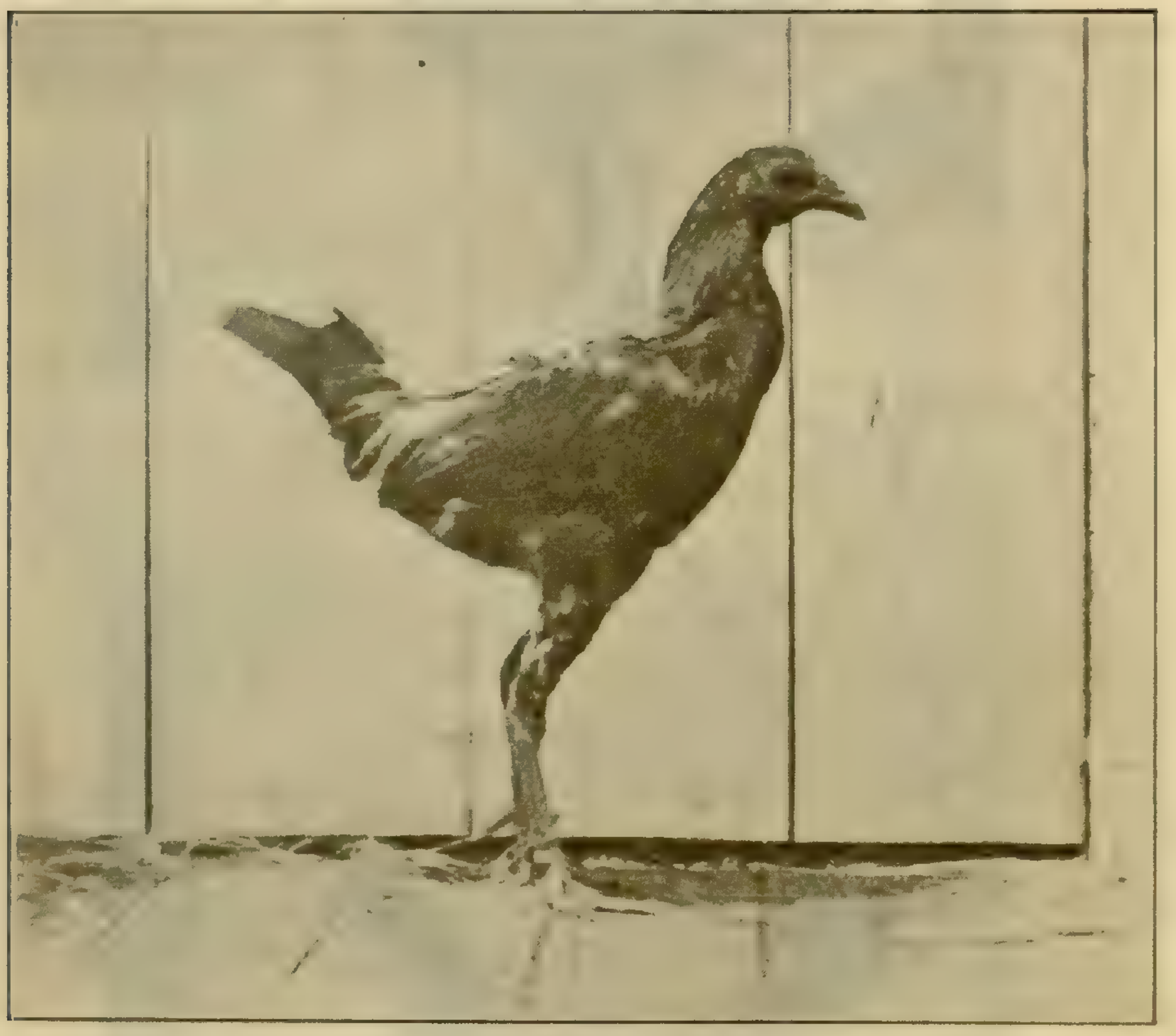

FIG. 4. Male, black-breasted game-bantam.

many surprises in store for us concerning *modes of distribution* of Mendelian factors. Moreover, the method by which crossing over of allelomorphic factors takes place is still in the speculative stage, so far as the cytological evidence is concerned, as are also many questions as to how the lineally arranged factors hold their order during the resting stages of the nucleus and during the condensed stages in the dividing chromosomes.

[8] The statement that I made in my recent book on the "Critique of the Theory of Evolution," that the *traditional* problem of heredity has been solved, is not in contradiction with the above statement which concerns the future problems of heredity.

FIG. 5. F_1 male, *a*, and female, *b*, out of Sebright by Game.

It does not seem to me to lessen in any way the importance of embryology to keep its problems for the present separated from those of the method of transmission of hereditary characters. It may well be that there are more important discoveries to be made in future in the field of embryology than in genetics, and that when the subject of chemical embryology has arrived at its goal it may be worth while to combine the two subjects into a single one. I am also aware that to many persons the interest in genetics is greatly increased when certain stages can be demonstrated through which the genes bring about their results. Far from being in opposition to such interests, I can illustrate this very point by a case of my own. The cock bird of the Sebright bantam is "hen-feathered" (Fig. 3*a*), *i. e.*, certain of the secondary sexual characters are like those of the hen (Fig. 3*b*). This is most noticeable in the short neck, back and saddle feathers as well as in the absence of the long tail feathers. When these birds are crossed to game bantams (a race in which the male has the usual secondary sexual characters, Fig. 4), the F_1 cocks are hen-feathered (Fig. 5*a*). This is true both when Sebright ♂ is crossed to game ♀ and when game ♂ is crossed to Sebright ♀. The latter cross shows that the dominant character is carried by the female Sebright as well as by the male.

When these F_1 birds are inbred, they produce in the next generation (F_2) both cock-feathered and hen-feathered males. There is complete segregation of the types that went into the cross. Whether one or two genes for hen-feathering are present is not entirely certain, but that Mendelian segregation occurs there can be no doubt.

I was led to see what would happen when the hen-feathered birds were castrated. Goodale had shown that when the hen of normal breeds is spayed, she develops the full male plumage, including the special feather regions in which the Sebright is hen-feathered. At the time of castration a few feathers were removed. The new ones that came in showed at once that a great change

had taken place both in the size, shape and color of the new feathers (Fig. 6), which became like those of the "normal" male. Since the F_1 birds were heterozygous,

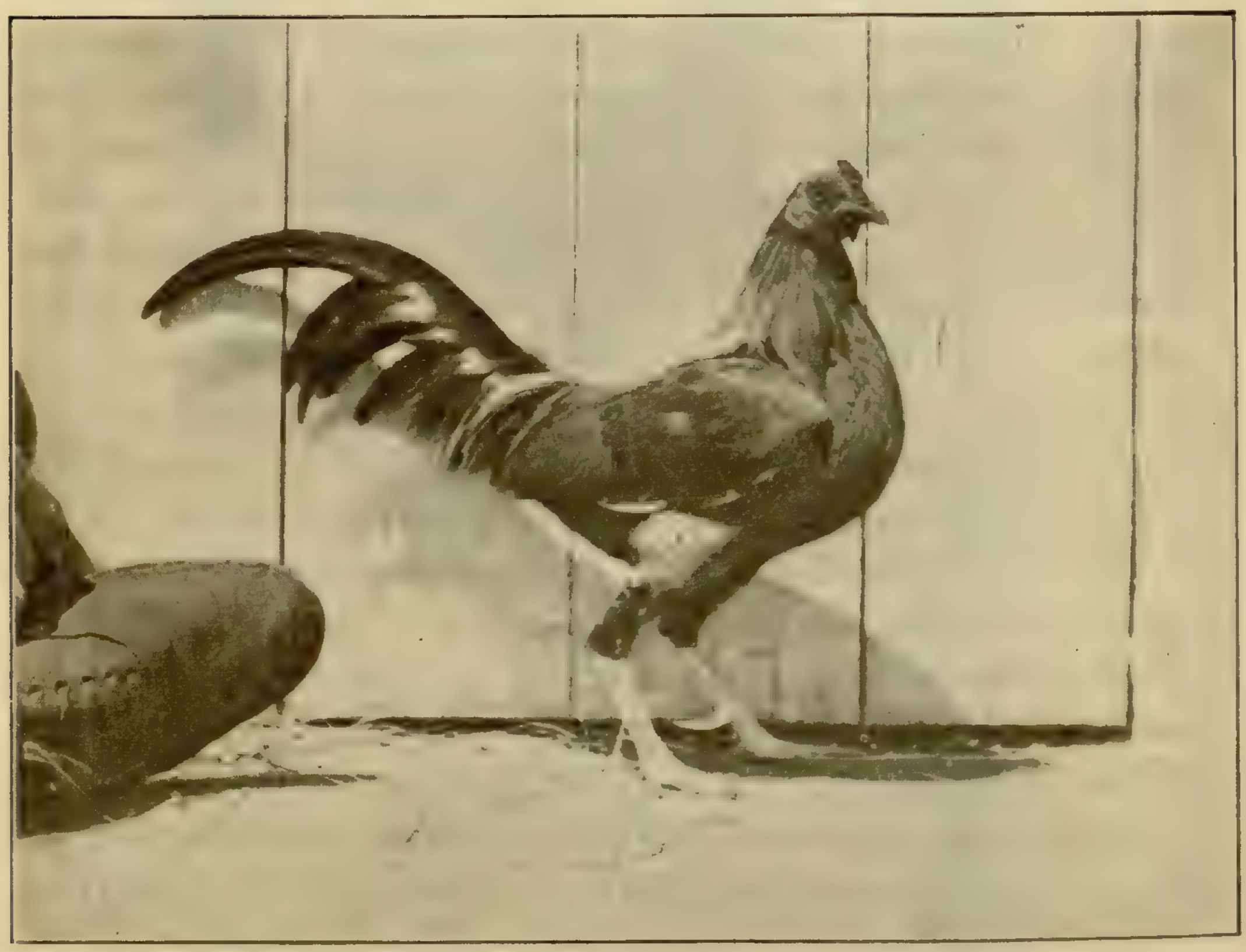

FIG. 6. Castrated F_1 male, originally like male in Fig. 5, *a*.

and the F_2 birds used might also have been heterozygous, it became important to castrate the Sebright males. This has been done and the same complete change takes place in them, as the accompanying figures of the birds (Fig. 7) and of a few of their feathers (Figs. 8 and 9) show very clearly.

Goodale's evidence from the spayed hen makes probable the view that the ovary of the hen produces some internal secretion that inhibits in her the full development of her plumage which is potentially the same as that of her male. After removal of the ovary the inhibition is removed and when the hen moults she develops her full possibilities of plumage. Similarly in the hen-feathered male, some internal secretion must inhibit the development of certain of the secondary sexual characters.

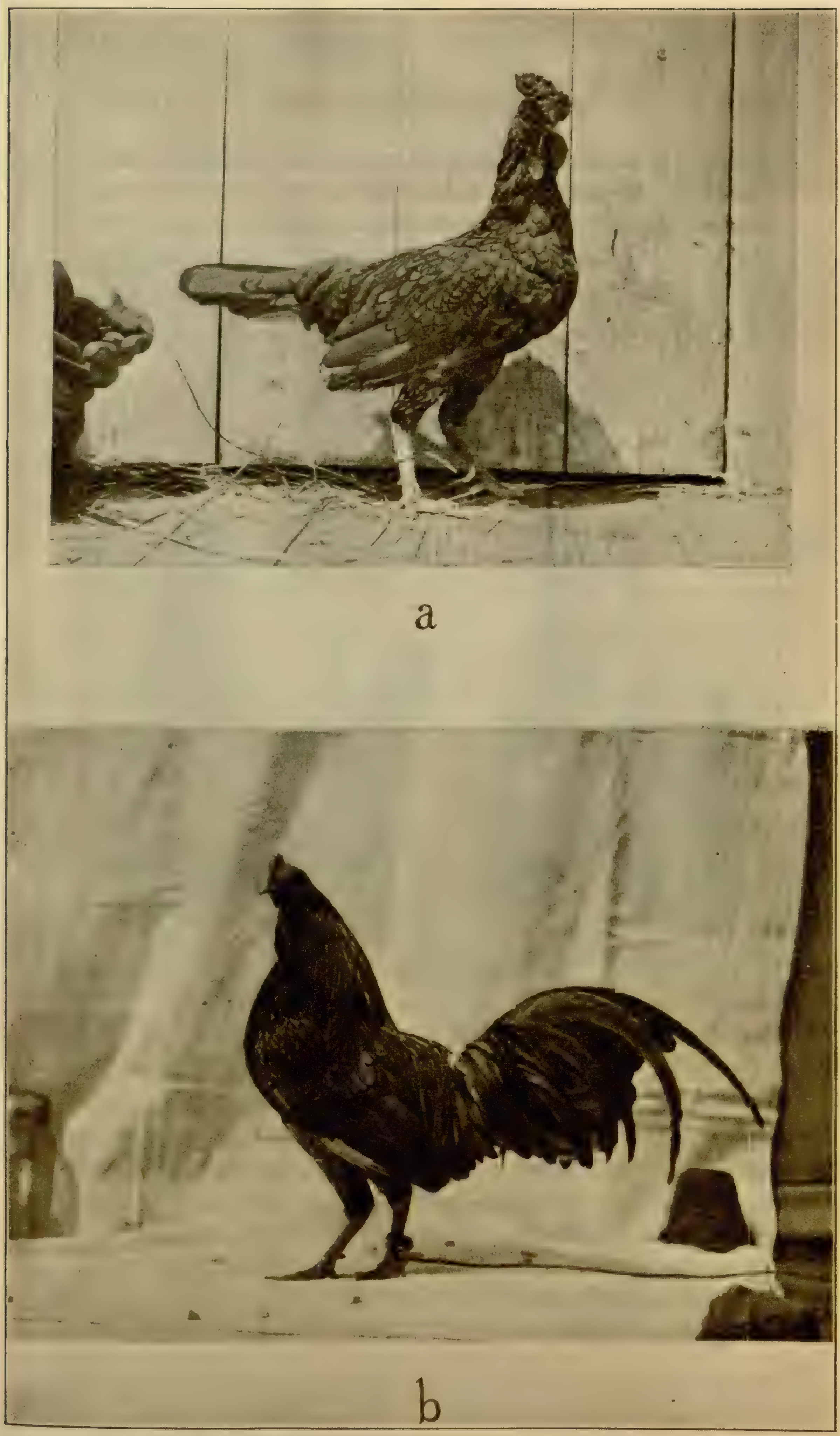

Fig. 7.—Schwight male, a, and similar bird after castration, b.

Here then we get an idea of one of the stages through which the products of Mendelian genes for hen-feathering produce their results. The presence of these genes within

FIG. 8. Feathers from F_1 cock (like that in Fig. 5*a*) before *a*, *b*, *c* and after *a'*, *b'*, *c'* castration; *a*, *a'* hackle, *b*, *b'* saddle, *c*, *c'* wing.

the male birds causes the testes to produce some substance that carried into the body inhibits the full development there of certain feathers. The presence of these genes in the other cells of the body is without influence on the plumage, except in the presence of the testes. The activity of the latter is such that a substance is produced there that has an inhibitory effect.

In other words, we are fortunate enough in this case

to be able to show a particular stage in the chain of events by which the character of certain feathers is influenced. I need not point out that there is not the slightest reason to identify the substance produced in the testes with the

FIG. 9. Feathers from Sebright cock (like Fig. 3*a* or Fig. 7*a*) before *a*, *b*, *c* and after *a′*, *b′*, *c′* castration; *a*, *a′* hackle, *b*, *b′* saddle, and *c*, *c′* wing.

substance of the gene; the chemical composition of the internal secretion may be entirely different from that of the gene, the latter producing its result in conjunction with substances resulting from other genes. There is every reason for supposing that the way in which the effects are produced here are the same as in all development when the end result is the collective product of substances pro-

duced by the hereditary genes—a single gene difference turning the scale in this way or in that. In this case we have, I think, an excellent illustration of the difference between the mechanism of inheritance and the chemical effects of genetic factors on development. Highly interesting and important as it undoubtedly would be to work out these connections, yet the evidence is very explicit in showing that the distribution of the materials of heredity during the maturation process of the egg and sperm is different in kind from their action through the cytoplasm on the developing organism.

For purposes, then, of closer analysis, it seems desirable in the present condition of genetics and embryology to recognize that the mechanism of distribution of the hereditary units or genes is a process of an entirely different kind from the effects that the genes produce through the agency of the cytoplasm of the embryo. The activity of the cytoplasm is, of course, bound up with the environment in which it takes place—a relation that is so intimate that in most cases the constitution of the cytoplasm and the nature of the environment in which it finds itself are studied as two sides of the same problem. It is true that the mechanism of Mendelian heredity may also be affected by the environment, certainly by the external environment, as Plough has shown for heat; and also probably by the cytoplasmic environment since Bridges has shown that the process is somewhat different in young and old flies. But there is no evidence that the relation of the maturation process to the environment is in any way related to the reactions that go on between the cytoplasm of the developing embryo and its environment, and it has only led to confusion whenever an attempt has been made to deduce from the nature of the embryonic reaction the nature of the mechanism that distributes the genes in heredity.

STUDIES ON INBREEDING. VII.—SOME FURTHER CONSIDERATIONS REGARDNG THE MEASUREMENT AND NUMERICAL EXPRESSION OF DEGREES OF KINSHIP[1]

DR. RAYMOND PEARL

1. In this series of studies certain concepts regarding the quantitative aspect of inbreeding have been presented. These concepts have in part been rigorously defined, and expressed in mathematical form. It is desirable to repeat here and extend in certain directions, the definition of two of the most fundamental of these concepts.

I. *Inbreeding* is defined in these studies as the condition or state in which an organism has in fact fewer different ancestors than the maximum number possible.

The degree or amount of inbreeding (total) is measured by a series of *inbreeding coefficients*, one for each ancestral generation, defined by the following equation:

$$Z_n = \frac{100\ (p_{n+1} - q_{n+1})}{p_{n+1}}\ , \qquad \text{(i)}$$

where p_{n+1} denotes the maximum possible number of different individuals involved in the matings of the $n+1$ generation, q_{n+1} the *actual* number of different individuals involved in these matings, and Z_n is the inbreeding coefficient for the $n+1$-th ancestral generation.

II. A state or condition of *relationship* or kinship between two organisms exists when these organisms have one or more common ancestors. The degree, intensity or closeness of the relationship is, in general, proportional to the number of different ancestors which the two individuals have in common, out of the whole number they might possibly have in common.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station. No. 113.

The degree or amount of relationship, in accordance with the above definition, is numerically measured by *relationship coefficients,* one for each ancestral generation. The coefficients are calculated in two slightly different ways according to whether they are being evaluated in connection with inbreeding coefficients, which will usually be the case, or independently.

A. When calculated in connection with inbreeding coefficients, a relationship coefficient is calculated, by methods presently to be shown by example, in accordance with the following equation:

$$\frac{K_n}{100} = \frac{(p_{n+1} - q_{n+1}) - ({}_sZ_{n-1} \cdot {}_sp_n + {}_dZ_{n-1} \cdot {}_dp_n)}{\frac{1}{2}p_{n+1}}, \qquad \text{(ii)}$$

where the letters have the same significance as in (i) with the additions that K denotes a relationship coefficient, a prefixed subscript s means that letters following it refer to the pedigree of the sire only, and a prefixed subscript d means that the letters following refer to the pedigree of the dam only.

B. When calculated independently of inbreeding coefficients, as, for example, to measure the relationship between two male animals, the relationship coefficient becomes

$$\frac{K_n}{100} = \frac{p_{n+1} - r_{n+1}}{\frac{1}{2}p_{n+1}}, \qquad \text{(iii)}$$

where $p_{n+1} - r_{n+1}$ denotes the number of ancestors in the $n+1$-th generation (each individual and its ancestry being counted once only) which occur, in the $n+1$-th or some earlier ancestral generation, in the pedigrees of both animals, or in other words which are common ancestors; p_{n+1} denotes the *total* number of ancestors in the same generation of both pedigrees taken together.

III. Inbreeding, defined in I, may exist in respect of any individual, as a result of any one or a combination of the following circumstances: (*a*) the sire of the individual has fewer than the maximum possible number of different ancestors, and no ancestors in common with the dam; (*b*)

the dam of the individual has fewer than the maximum possible number of different ancestors, and no ancestors in common with the sire; or, (*c*) the sire and dam have a certain number of common ancestors, and hence are, in the common sense of the word, related to each other in some degree.

IV. We may separate conceptually that portion of the total inbreeding due to *a* or *b* or any combination of *a* and *b*, from that portion of the total inbreeding due to *c*, and define as due to *relationship* between the sire and dam that amount or degree of inbreeding (in the sense of I) which remains after the amount due to *a* or *b* (of III) or any combination of *a* and *b* has been subtracted from the total inbreeding.

A numerical expression of the portion of the inbreeding in the *n*th generation due to relationship is obtained by a *partial inbreeding index* of the following form:

$$KZ_n = \frac{50(K_n)}{Z_n}. \qquad \text{(iv)}$$

Expressed in words this means that we take as an index of the part of the inbreeding due to relationship the percentage which one half of the relationship coefficient is of the inbreeding coefficient, both referred of course to the same ancestral generation.

2. The above paragraphs define a relationship coefficient much more rigorously and generally than was done in my earlier paper on the subject,[2] or in "Modes of Research in Genetics."[3] Not only is this a gain in itself, but also it makes possible a great simplification in the actual work of calculating coefficients of relationship from pedigrees. Extensive experience has shown that the method of making these determinations given in my earlier paper left much to be desired in the direction of simplicity, ease of application, and even of accuracy in case the pedigree dealt with was at all complicated in

[2] Pearl, R., AMER. NAT., Vol. XLVIII, pp. 513–523, 1914.

[3] Pearl, R., "Modes of Research in Genetics," New York, 1915 (Macmillan & Co.). Cf. pp. 101–156.

respect of the distribution of its ancestral repetition. Out of actual laboratory experience has been developed the more simple and rigorous analysis of the matter presented in this paper.

3. It would appear that the briefest and simplest way to make clear our concept of kinship measurements, its use in the analysis of inbreeding, and its practical application to pedigrees, is to carry out the work on some concrete examples, given by actual pedigrees showing a rather high degree of inbreeding or relationship. This we shall accordingly proceed at once to do, taking as our first example the pedigree through five ancestral generations of the Jersey cow Letty's Fancy Lady (241551).

The pedigree (for five ancestral generations) of this cow is presented in Tables I and II. Table I gives the pedigree of her *sire,* Rioter's St. Lambert King (58644), and Table II gives the pedigree of the *dam* of the cow, Letty's Fancy (160320). Tables I and II together, therefore, give the complete pedigree (to the extent already indicated) of the cow herself. The reason for splitting the pedigree into two parts in this way in its presentation will be apparent as we proceed. The numbers preceding the names of the animals are the registry numbers in the Herd Books of the American Jersey Cattle Club.

In Tables I and II the symbols have the following significance: A solid circle indicates a primary reappearance of an ancestor, having reference to the pedigree of Letty's Fancy Lady *as a whole,* and an open circle indicates an entailed reappearance consequent upon the primary reappearance denoted by the solid circle. A solid square indicates a primary reappearance in the pedigree of the *sire* of Letty's Fancy Lady, *considered by itself and without reference to her dam's pedigree;* an open square denotes reappearance consequent upon those indicated by the solid squares. Finally, a solid diamond indicates a primary reappearance of an ancestor in the pedigree of the *dam* of Letty's Fancy Lady, *considered by itself,* while the open diamonds denote the corresponding entailed reappearances.

TABLE I

PEDIGREE OF RIOTER'S ST. LAMBERT KING (58644), SIRE OF LETTY'S FANCY LADY (241551)

Ancestral Generation [4]

1	2	3	4	5
Rioter's St. Lambert King No. 58644 ♂	Rioter of St. Lambert No. 16501 ♂	King of St. Lambert	No. 15175 ♂ ●■ King of St. Lambert	No. 13656 ♂ Ida's Rioter of St. Lambert	No. 4558 ♂ ● Bachelor of St. Lambert
					No. 24990 ♀ Ida of St. Lambert
				No. 24991 ♀ Allie of St. Lambert	No. 2238 ♂ ●■ Stoke Pogis 3d
					No. 5122 ♀ Kathleen of St. Lambert
			No. 28353 ♀ May Day Stoke Pogis	No. 2238 ♂ Stoke Pogis 3d	No. 1259 ♂ Stoke Pogis
					No. 3239 ♀ Marjoram
				No. 5109 ♀ May Day of St. Lambert	No. 1066 ♂ Lord Lisgar
					No. 1373 ♀ Jerne
	Phyllis of St. Lambert No. 78867 ♀	Allie of St. Lambert 2d	No. 43671 ♀ Allie of St. Lambert 2d	No. 8388 ♂ Canada's John Bull	No. 6036 ♂ Sir George of St. Lambert
					No. 12968 ♀ Nymph of St. Lambert
				No. 24991 ♀ ●■ Allie of St. Lambert	No. 2238 ♂ ○□ Stoke Pogis 3d
					No. 5122 ♀ ○□ Kathleen of St. Lambert.

[4] Referred to the propositus, Letty's Fancy Lady (241551).

TABLE II

PEDIGREE OF LETTY'S FANCY (160320), DAM OF LETTY'S FANCY LADY (241551)

Ancestral Generations

1	2	3	4	5
Sex ♀	♂	No. 13657 ♂ Exile of St. Lambert	No. 4558 ♂ Bachelor of St. Lambert	No. 3143 ♂ Orloff
				No. 6638 ♀ Charity of St. Lambert
			No. 24991 ♀ ● Allie of St. Lambert	No. 2338 ♂ ○ Stoke Pogis 3d
				No. 5122 ♀ ○ Kathleen of St. Lambert
		No. 73475 ♀ Letty Rioter	No. 10481 ♂ Diana's Rioter	No. 6036 ♂ ● Sir George of St. Lambert
				No. 6636 ♀ Diana of St. Lambert
			No. 48128 ♀ Letty Coles 2d	No. 10481 ♂ ● ◆ Diana's Rioter
				No. 23351 ♀ Letty Coles
Letty's Fancy	♀ No. 48228 Riot	No. 17408 ♂ St. Lambert Boy	No. 8388 ♂ ● Canada's John Bull	No. 6036 ♂ ○ Sir George of St. Lambert
				No. 12968 ♀ ○ Nymph of St. Lambert
			No. 14880 ♀ Oakland's Nora	No. 5248 ♂ Lorne
				No. 5123 ♀ Pet of St. Lambert
	Lady Letty Rioter	No. 124201 ♀ Lady Letty Lambert	No. 17408 ♂ ● ◆ St. Lambert Boy	No. 8388 ♂ ○ ◇ Canada's John Bull
				No. 14880 ♀ ○ ◇ Oakland's Nora
			No. 48128 ♀ ● ◆ Letty Coles 2d	No. 10481 ♂ ○ ◇ Diana's Rioter
				No. 23351 ♀ ○ ◇ Letty Coles
No. 160320	No. 142135			

With these data in hand we may proceed to the evaluation first of the total inbreeding. We have in Table III the pedigree elimination table for this purpose, which lists the primary reappearances indicated by solid circles.

TABLE III

PEDIGREE ELIMINATION TABLE FOR THE TOTAL INBREEDING OF LETTY'S FANCY LADY

Name of Animal Primarily Reappearing	Ancestral Generation in which Primary Reappearance Occurs				
	1	2	3	4	5
King of St. Lambert	—	—	1	2	4
Allie of St. Lambert	—	—	—	2	4
Canada's John Bull	—	—	—	1	2
St. Lambert Boy	—	—	—	1	2
Letty Coles 2d	—	—	—	1	2
Bachelor of St. Lambert	—	—	—	—	1
Stoke Pogis 3d	—	—	—	—	1
Sir George of St. Lambert	—	—	—	—	1
Diana's Rioter	—	—	—	—	1
Totals	0	0	1	7	18

Whence, by the usual method, using the tables of Pearl and Miner,[5] we have the following values:

TOTAL INBREEDING COEFFICIENTS FOR LETTY'S FANCY LADY

$$Z_1 = 0, \quad Z_2 = 12.50, \quad Z_3 = 43.75, \quad Z_4 = 56.25.$$

Let us next consider Table IV, which gives the pedigree elimination for the pedigree of the sire, as given in Table I, considered by itself, the primary reappearances listed being those indicated by solid squares. It must be particularly noted that the primary reappearances listed in this table are referred to the ancestral generations of the pedigree of Letty's Fancy Lady, and *not* to the pedigree of Rioter's St. Lambert King, her sire, with whose pedigree we are dealing.

TABLE IV

PEDIGREE ELIMINATION TABLE FOR RIOTER'S ST. LAMBERT KING

Name of Animal Primarily Reappearing	Ancestral Generation[6] in which Primary Reappearance Occurs				
	1	2	3	4	5
King of St. Lambert	—	—	1	2	4
Allie of St. Lambert	—	—	—	1	2
Stoke Pogis 3d	—	—	—	—	1
Totals	0	0	1	3	7

[5] Pearl, R., and Miner, J. R., Maine Agr. Expt. Stat. Ann. Rept. for 1913, pp. 191–202.

[6] Referred to the pedigree of Letty's Fancy Lady.

In Table V exactly corresponding data are given for the pedigree of Letty's Fancy, the dam of Letty's Fancy Lady. The primary reappearances here are those indicated by solid diamonds in Table II.

TABLE V

PEDIGREE ELIMINATION TABLE FOR LETTY'S FANCY

Name of Animal Primarily Reappearing	Ancestral Generation[6] in which Primary Reappearance Occurs				
	1	2	3	4	5
St. Lambert Boy	—	—	—	1	2
Letty Coles 2d	—	—	—	1	2
Diana's Rioter	—	—	—	—	1
Totals	0	0	0	2	5
Combined Totals of Tables IV and V	0	0	1	5	12
Difference between combined totals, and totals of Table III (total inbreeding)	0	0	0	2	6

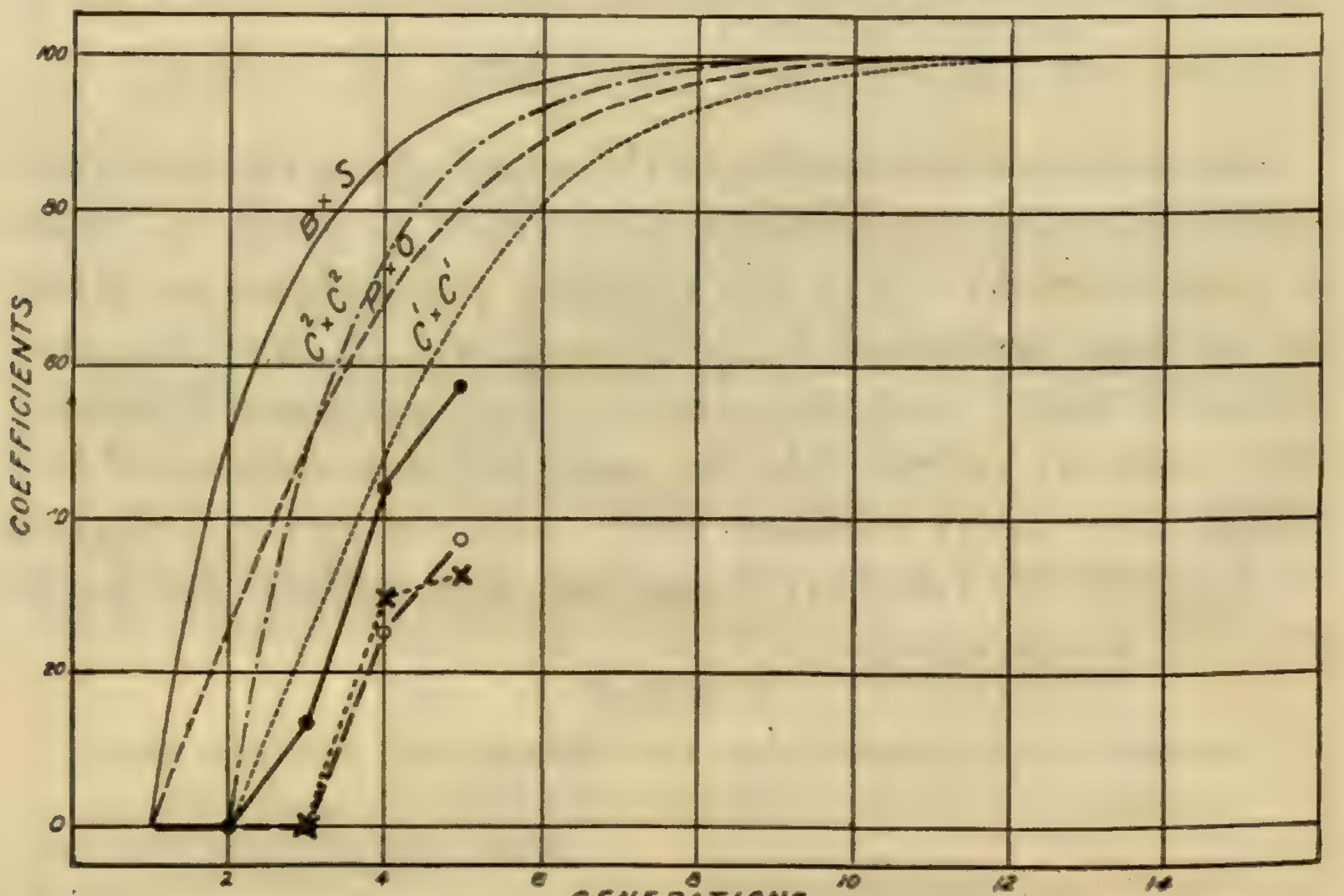

FIG. 1. Diagram showing the inbreeding and relationship curves for Letty's Fancy Lady. Total inbreeding coefficients—solid line and circles; relationship coefficients—dash line and open circles; partial inbreeding coefficients—dotted line and crosses. The smooth curves at the top of the diagram are the total inbreeding curves for continued brother × sister, parent × offspring, and single and double cousin × cousin mating. These are inserted for comparison.

From the last line of Table V we deduce *relationship coefficients* as follows:

$$K_1 = 0, \qquad K_3 = \frac{100 \times 2}{8} = 25.00,$$

$$K_2 = 0, \qquad K_4 = \frac{100 \times 6}{16} = 37.50.$$

Expressed in words these coefficients mean that Rioter's St. Lambert King and Letty's Lady are related to the amount or degree of 25 per cent. in the third, and 37.5 per cent. in the fourth ancestral generation.

In Fig. 1 are shown the total inbreeding (solid line and dots), the relationship (dashes and open circles), and the partial inbreeding (dots and crosses) curves for Letty's Fancy Lady.

Finally we have, from (iv), the following coefficients of partial inbreeding due to relationship.

$$KZ_1 = \frac{50\,(0)}{0} = 0,$$

$$KZ_2 = \frac{50\,(0)}{12.5} = 0,$$

$$KZ_3 = \frac{50\,(25)}{43.75} = 28.57,$$

$$KZ_4 = \frac{50\,(37.5)}{56.25} = 33.33.$$

We thus see that of the total inbreeding observed in the third ancestral generation of Letty's Fancy Lady, none is due to relationship between her sire and dam; of that observed in the fourth ancestral generation, 28.57 is due to such relationship; and finally, of that observed in the fifth ancestral generation, one third arises because of relationship between sire and dam.

4. Let us next consider an example of measuring relationship independently, altogether apart from consideration of inbreeding. We may take a very simple case afforded by the two milking shorthorn cows, Imp. Milk

Maid 211032, and Imp. White Queen 545726. The pedigrees of these animals follow in Tables VI and VII. The problem before us is to measure and express numerically the degree of relationship or kinship between these two animals.

TABLE VI

PEDIGREE OF IMP. MILK MAID (211032)

		Ancestral Generations	
	1	2	3
Sex ♀	♂	No. 409267 ♂ Morning Sun	No. 409193 ♂ Inspector
			No. —— ♀ Bessie 44th
	No. 425404 Ireby Signet	No. —— ♀ Tulip 28th	No. 409093 ♂ Dainty Bean
			No. —— ♀ Tulip 23d
Imp. Milk Maid	♀	No. 433648 ♂ Border Stamp	No. 80356 ♂ Arkin Beau
			No. —— ♀ White Sunshine
No. 211032	No. 211017 Border Lady	No. —— ♀ Lady Balmoral	No. 425402 ♂ Balmoral Pearl
			No. —— ♀ Lady Benedict's Farewell

We see that in these two pedigrees there is, in the first ancestral generation, one ancestor (Ireby Signet) which occurs in both. Hence we have

$$K_1 = \frac{100\,(1)}{2} = 50.00.$$

In the second generation there are three ancestors (Morning Sun, Tulip 28th and Border Stamp), which occur in both pedigrees, whence it follows that

$$K_2 = \frac{100\,(3)}{4} = 75.00.$$

In the third generation there are six common ancestors

TABLE VII

PEDIGREE OF IMP. WHITE QUEEN (545726)

		Ancestral Generation		
		1	2	3
Sex ♀		♂	No. 409267 ♂ Morning Sun	No. 409193 ♂ Inspector
				No. ——— ♀ Bessie 44th
Imp. White Queen	No. 425404 Ireby Signet		No. ——— ♀ Tulip 28th	No. 409093 ♂ Dainty Bean
				No. ——— ♀ Tulip 23d
No. 545726		♀	No. 433648 ♂ Border Stamp	No. 80356 ♂ Arkin Beau
				No. ——— ♀ White Sunshine
	No. 501778 Border Queen		No. ——— ♀ Diamond Queen	No. 501767 ♂ Levens Guardsman
				No. ——— ♀ Landford Diamond

(all involved from the second ancestral generation) and hence

$$K_3 = \frac{100\,(6)}{8} = 75.00.$$

So that we may say that Imp. Milk Maid and Imp. White Queen are 50 per cent. related in the first ancestral generation, and 75 per cent. in the second and third. This case will illustrate the superiority of the present exact numerical expression of relationship over the ordinary verbal expression. These two cows are half sisters, both having the same sire (this degree of relationship is indicated numerically always by $K_1 = 50$). But they are more closely related than two individuals which are *only* half sisters, because they have *also* one grandsire (Border Stamp) in common. Their total degree of relationship is simply not expressible verbally, by any term of kinship known to me in the English language. Yet by the method

here described it is exactly expressible in the form $K_1 = 50$, $K_2 = K_3 = 75$.

5. It will be perceived that the form of relationship coefficient here proposed leads to precisely the same numerical results in simple pedigrees, with not too involved inbreeding or kinship, as that given in my former paper[7] except for the fact that I have here changed the subscript designation of the K's to bring them into conformity with the total inbreeding coefficients. The earlier form proposed for these coefficients would always give the same numerical values as the present one if certain rather complicated rules of application, which were not clearly or rigorously set forth in the earlier paper, were to be followed. But the present simplified form does away entirely with the need for these complicated rules of procedure.

6. It is of interest to set forth in tabular form the values of the relationship coefficients for the commonly recognized degrees of kinship. This is done in Table VIII, in which the different degrees of kinship are arranged in descending order of closeness, in general. In some cases, as, for example, parent and offspring and half brothers (or half sisters), groups of two or three different sorts of kinship showing the same numerical degree of relationship should be regarded as bracketed, since there is no more reason for placing one of these first than another.

From this table a number of interesting points emerge. We note that the absolute maximum of closeness of relationship is that of brother and sister. The parent and offspring relationship is one half as close. Uncle and nephew (or niece), or single first cousins, are twice as closely related as grandparent and offspring. Some of these comparisons made obvious by the table may seem at first thought to give unexpected results, but if one will take the trouble to write down pedigrees for the stated

[7] Pearl, R., AMER. NAT., Vol. XLVIII, pp. 513–523, 1914.

degree of kinship, he will see upon careful consideration the reasonableness of the numerical result.

TABLE VIII

VALUES OF THE RELATIONSHIP COEFFICIENTS FOR VARIOUS DEGREES OF KINSHIP

Degree of Kinship	K_1	K_2	K_3	K_4
Brother and brother (or sister)	100	100	100	100
Parent and offspring	50	50	50	50
Half-brother and half-brother (or half-sister)	50	50	50	50
Double first cousins	0	100	100	100
Single first cousins	0	50	50	50
Uncle and nephew (or niece)	0	50	50	50
Grandparent and offspring	0	25	25	25
Quadruple second cousins	0	0	100	100
Double second cousins	0	0	50	50
Single second cousins	0	0	25	25
Propositus and first cousin once removed	0	0	25	25
Propositus and first cousin twice removed	0	0	0	12.5

7. There are two points in the development of relationship coefficients in this paper which may seem open to criticism. The first is that according to the definitions and formulæ of this paper, the degree of relationship between two individuals is not affected by the number of times *the same* common ancestor occurs in the pedigree of either of the two individuals. The fact that such ancestor occurs at least *once* in both pedigrees makes it a common ancestor. If it occurred more times it would not be a *more* common ancestor, because after all it would still be, all the time, just the same identical individual, made up of the same germ plasm. Put in another way, it is *community* of ancestry of two individuals which makes kinship. But the multiple appearance of the *same* individual in two pedigrees does not make any more ancestors common to the two related individuals than if this ancestor occurred only once in each pedigree. Consider an individual A which is rather intensely inbred with reference to an ancestor X. Consider another individual B which is also inbred to some extent with reference to the same individual X. Because they have a common ancestor X, A and B are related. But, according to the concep-

tion on which the present method of measuring kinship is based, the fact that A and B happen both to be inbred in respect to X, does not make them any more closely related to each other than if they were not so inbred. It may be of interest in this connection to point out, not as adding to the scientific exactitude of the position here taken, but as indicating what the common sense of men who have given thought to the subject of consanguinity has been, that the position here adopted that in determining degree of kinship a common ancestor counts but once as such, appears to be exactly in agreement with the position of both the canon law and the civil law on the same point.

The second point in regard to which criticism might seem to be possible is the method of referring the inbreeding or relationship to the ancestral generations. In all of these Studies the inbreeding or relationship is referred to the generation of the more remote (from the propositus) of the two appearances in a pedigree of a repeated ancestor. The logic of this procedure, rather than the reverse, is found in the circumstance that the fact of inbreeding (or kinship) does not establish itself until the more remote reappearance is reached. Thus it is impossible to know that a mating is of uncle and niece until the grandparental generation is reached.

$$a \begin{cases} b \\ \\ c \end{cases}$$

$$x \begin{cases} d \begin{cases} b \\ \\ c \end{cases} \\ \\ e \end{cases}$$

Ancestral generation...	1	2

a is the uncle of x, the common ancestors being b and c, but this fact is not known until the second ancestral generation is reached. The only logical method of representing these facts exactly in a numerical way would seem to be to say, in effect, that up to and including the first ancestral generation of a and x there is no evidence that these individuals are at all related, and therefore

$K_1 = 0$. In the second ancestral generation, on the contrary, it appears that two ancestors, b and c, in the pedigree of x are the same individuals as appeared in the first ancestral generation of a. Therefore it now appears that a and x are related to the extent of 50 per cent. by the existence of community of ancestry in the second ancestral generation. It would seem only logical to attach the numerical measure of relationship to the generation in which it is first proved to exist. Again, this is precisely the point of view regarding the matter which has been taken by the canon law and Roman civil law.

These two points, which seem so obvious to the writer as to be difficult to discuss, are taken up here because correspondence has shown that they have been a source of difficulty with some who have undertaken the study of inbreeding in domestic animals by the methods set forth in these studies. It is hoped that the simpler and more precise definitions of both inbreeding and relationship constants given in this paper may help to clear up such difficulties, which must arise, it would seem, from a lack of a thorough grasp of the characteristics of pedigrees.

Summary

In this paper the basic concepts of inbreeding are redefined in a simple and rigorous manner, and on the basis of these definitions a new and more accurate method of measuring and expressing numerically the degree of kinship between any two individuals whatsoever, whose pedigrees are known, is set forth and illustrated by examples.

A new constant, the *partial inbreeding index*, is described. Its purpose is to indicate numerically the part of the total inbreeding exhibited in the pedigree of any individual which is due to relationship between the sire and the dam of that individual.

MULTIPLICATION BY FISSION IN HOLOTHURIANS[1]

DR. W. J. CROZIER

THERE is to be found in various text-books the statement that certain pedate holothurians are capable of spontaneous transverse division, each part so formed producing a new individual (Lang, 1894, p. 1095; Morgan, 1901, p. 144). This opinion seems to be based, so far as I can learn, upon the observations of Dalyell (1851, p. 74; Pl. XIV), although Morgan says that "more recent observers have confirmed this discovery." Chadwick (1891), also, found small individuals of *Cucumaria planci* to undergo self-division, and in one instance the posterior portion so formed also divided. These are records of division in holothurians which were being kept in small aquaria, and there has been no evidence, so far as I am aware, going to show that self-division of adult pedate holothurians is a method of propagation among these animals in their normal surroundings. Hence the possibility of non-sexual reproduction in this way is usually stated with reserve (cf. Lang, 1894).

In other classes of echinoderms (aside from echinoids) the expedient of reproduction by fission is of course not unusual; but in ophiuroids and in such starfishes as *Linckia* (Clark, 1913) and *Coscinasterias* (Crozier, 1914),[2] we are dealing with the division or fragmentation of a

[1] Contributions from the Bermuda Biological Station for Research, No. 66.

[2] I have been able to secure further evidence regarding the fragmentation of *Coscinasterias* (*Asterias*) *tenuispina* (Lam.), which proves conclusively that the rules previously deduced (Crozier, 1915*a*) regarding this process are indeed valid. This evidence will be published in connection with a description of experiments on the direction of progression in *Coscinasterias*. The presence of great variation in ray-length, as well as of a variable number of madreporites, gives an opportunity to test out in this species the validity of certain ideas concerning "physiological polarization" in asteroids.

many-armed creature relatively deficient in morphological centralization; whereas in *Cucumaria* and in *Holothuria* the body is compactly built, the animal much more of a unified individual. Consequently the self-division of these holothurians is not without interest, especially since in these cases the plane of separation is anatomically perpendicular to that employed among the astroradiates, and it is the purpose of this paper to present evidence which proves that adult specimens of at least one species, *Holothuria surinamensis* Ludw., do as a matter of fact divide transversely into two parts under conditions which must be regarded as normal. Since these divisions are not infrequent in large numbers of specimens, if not in a single life-history, we must conclude that fission represents a regular means of mutliplication in this species.

A few years ago I found (Crozier, 1915*b*) that about 10 per cent. of the examples of *H. surinamensis* which were studied showed a condition of either the oral or of the cloacal end which—on the basis of observed regenerations following experimental cutting—I interpreted as representing regeneration, possibly as a consequence of spontaneous self-division. Similar conditions have been noted by others for some other species of holothurians, *e. g.*, by Benham (1912, p. 136) for *Actinopyga* (*Mülleria*) *parvula* (Selenka), but they have usually been referred to regeneration after injury by such bottom feeding fishes as small sharks. Dr. H. L. Clark informs me that he has found a corresponding state of affairs in some Australasian holothurians, at least in regard to the occurrence in nature of specimens showing posterior regeneration.

I subsequently obtained young individuals of *H. captiva* Ludw., about 6 mm. in length, which were observed to divide spontaneously in the laboratory (Crozier, 1914, p. 18), precisely according to the procedure figured by Dalyell (1851) and by Chadwick (1891). Only a single adult *H. captiva* has been discovered, however, in which there was evidence of normal regeneration; this indi-

vidual, which was 40 mm. long, was obtained among a group of 47 taken from under a large rock in April, 1916.[3] The anterior end for a distance of 7 mm. from the tip was light greenish yellow, with ten very feebly developed tentacles; there was a sharp line of demarcation between the light yellow surface and the dark olive green of the rest of the body. If *H. captiva* undergoes division normally, it can only occur in very young stages.

With *H. surinamensis*, however, the case is quite different. In Table I there are summarized results of the examination of several series of these animals collected at different times for this particular purpose. It will be noted that in these collections from 2.5 to 16.9 per cent. (on the average about 11 per cent.) of the individuals show a condition of either the oral or of the cloacal end which is interpreted as representing regeneration. This seems to be about the proportion of such instances which is to be met with in general collecting, although numerical records have been kept only in the cases cited. The specimens represented in the tabulation were obtained

TABLE I

THE RELATIVE NUMBER OF CASES IN WHICH *Holothuria surinamensis* WAS FOUND TO BE REGENERATING IN NATURE

Date	No. examined	Regenerating			
		Oral	Cloacal	Total	%
June, July, 1913.....	200	7	13	20	10.0
July 30, 1916	39	0	1	1	2.5
Aug. 26, 1916.....	84	6	5	11	13.1
Jan. 25, 1917.....	70	6	3	9	12.8
Jan. 31, 1917..	53	4	5	9	16.9
Total.....	446	23	27	50	11.2

Ratio of regenerating oral to cloacal ends = 1 : 1.17.

from one locality, Fairyland Creek, where they notably abound; but the species has been collected at many other stations, where also the regenerating individuals are to be found in approximately the same proportion. The

[3] It is known that some holothurians tend to congregate together in considerable numbers at their time of breeding. Graber considered this to indicate the presence of a chemical sense (Delage et Hérouard, 1903).

season of the year seems not to influence the occurrence of regenerating specimens.

The criterion of regeneration in these cases consists in the presence of an anterior or posterior terminal part of the body characteristically different in appearance from the normal buccal or cloacal end, the surface being clearly marked off from that of the rest of the body. In typical examples these regenerated ends of the animal are more sharply pointed than is usual; they bear feebly developed tentacles (at the anterior end), tube feet, and dorsal papillæ, which are less reactive than those on animals judged to be not regenerated; and these appendages are very lightly pigmented (Fig. 1). These characteristics

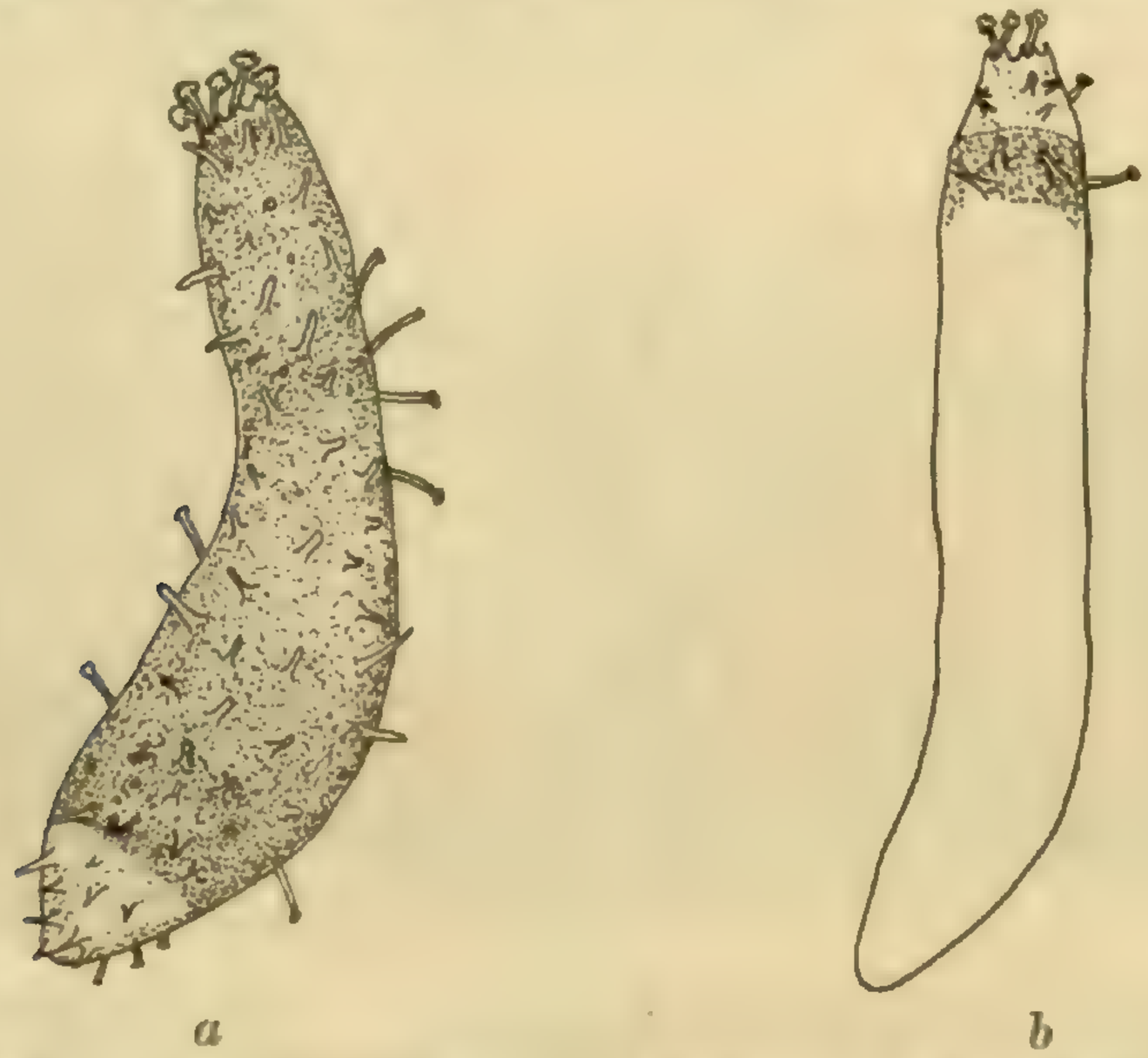

FIG. 1. Regeneration found occurring naturally. Semidiagrammatic sketches, showing differences in pigmentation of *a*, cloacal end, *b*, oral end. Natural size.

undoubtedly become less prominent with time, the coloration tending, however, to remain pale on the ventral surface (trivium). The first pigment to appear is the green fluorescent one (cf. Crozier, 1914, p. 9 and 1915*b*); the dark brown substance develops more slowly, just as in the growth of the post larval holothurians of this and related species. The spicules of the podia and skin seem fewer than in corresponding non-regenerating parts, but are of the usual sizes and shapes. The tentacles on regenerating buccal ends are always fewer (9–15) than on the normal individual (20).

That the oral and cloacal terminations just described do in reality represent regeneration, has been verified by observation of the course of regeneration in the laboratory after experimentally cutting the holothurians in various ways (cf. Crozier, 1915*b*). Certain specimens also have been tabulated as "regenerating" when their appearance (Fig. 2, *c*), backed up by dissection, suggested that they had just completed division and had not yet begun to regenerate. These specimens lacked either a cloaca, or the stone ring and buccal structures, depending, obviously, on their former relation to the complete individual from which they were derived.

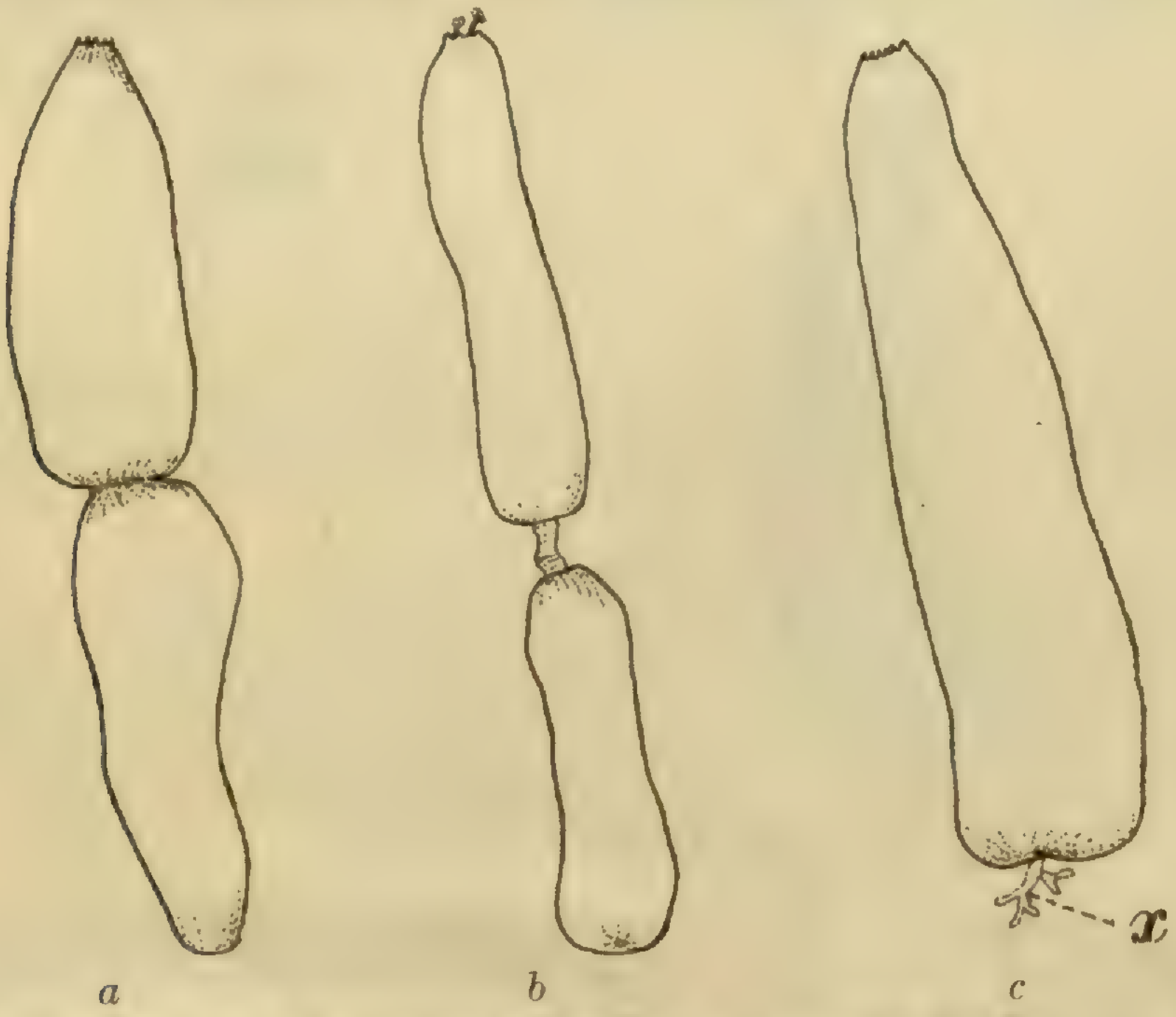

FIG. 2. Outlines of individuals dividing or recently divided. In *a* and *b* the division is shown in progress, × ½ ; in *b* the exposed portion of the undivided intestine is visible; in *c* is given the outline of a recently separated oral half, natural size. At *x* is seen a protruding bit of the portion of the respiratory trees retained within this half animal. The characteristic puckering of the surface at the division area is also indicated.

The evidence that the regeneration found occurring under natural conditions results from the self-division of adult holothurians, involves two considerations. The first concerns (*a*) the relative size of the regenerating animals, and (*b*) the relative frequency of anterior and posterior ends noted as regenerating. The second has to do with direct observations of self-division.

One hundred *H. surinamensis* collected in Fairyland Creek ranged in length from 6 to 18 cm., with the mode at 14 cm. The regenerating specimens ranged in length from 4 to 9 cm., with the mode at 7 cm. While no numerically exact argument can be based on these figures, since the length of any one holothurian is variable, the fact does stand out that the regenerating animals are about one half the length of the non-regenerating ones. There is also the significant fact that not a single instance has been found in which both a (supposedly) new oral and new cloacal end were present. If self-division has occurred, then we should expect to find new oral and new cloacal extremities in equal frequency; among the rather small number of cases available, we find their ratio to be as 1:1.17, an agreement sufficiently close to favor belief in self-division.

The evidence concerning the second point is even more conclusive. I have seen, in all, nine cases in which a holothurian (*H. surinamensis*), in the laboratory, divided itself into two parts. The animals concerned seemed healthy, and bore no visible signs of having been in any way injured. In no case did the halves so formed redivide, although in two cases the resulting portions lived in the laboratory for a month (Aug. 3 to Sept. 5, 1916), during which time, even in the absence of food, missing structures were regenerated.

In one case the process of division occupied five days; in another, twenty-four hours. Probably it is executed more rapidly in the field. The details of the division were not notably different from those described by Chadwick (1891) for *Cucumaria,* except perhaps in one particular. The intestine is not drawn out between the separated halves, as found in *Cucumaria* and as I have observed in the young of *H. captiva.* Division begins midway of the length of the body with a deep insinking of the "dorsal" bivium. A powerful circular constriction, accompanied by some slight local disintegration of the integument, completes the separation (Fig. 2). During the progress of division the animal is quiescent, although it

may be adhering firmly by its tube feet to the vertical wall of the aquarium. When the constriction and separation of the skin- and muscle-layers is completed, a short length of the intestine usually remains for a time connecting the two pieces; it may rupture close to one of them, or may disintegrate completely. The point to be noted is, that the resultants of the division do not move apart, but remain quiescent.

Lastly, after considerable searching, I found in the field one case in which division had evidently just been completed. The halves were still joined by an exposed portion of the gut.

On the basis of all this evidence there is certainly reason to believe that *Holothuria surinamensis*, in the adult state, normally multiplies its numbers by a process of binary fission. The resulting organisms readily complete their missing parts, but probably do not undergo a second division until after the lapse of a considerable interval, if they do at all.

It would be of some interest to determine the nature of the sexual products in the animals which thus result from division.

AGAR'S ISLAND,
BERMUDA

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SHORTER ARTICLES AND DISCUSSION

AN ATTEMPT TO MODIFY THE GERM PLASM OF ŒNOTHERA THROUGH THE GERMINATING SEED

When a new character appears in a homozygous race or species it may be either a mutation or an acquired character. If a mutation it has been produced because the germ plasm has in some way been affected and the succeeding generations may be expected to show the same variation. If an acquired character it will be present for a single generation and is then lost unless the cause that produced the somatic change also modified the germ plasm in such a manner that it may develop the same character, in succeeding generations. By "acquired characters" is meant any and all changes that are wrought in the soma of the organism by the environment considered in its broadest sense. It is the creed of modern biology that acquired characters are not inherited unless the environmental influences also play on the germ cells even while focused on the body tissues, producing at the same time potential alterations in the former and visible changes in the latter.

The causes of mutation are in dispute. It is fairly obvious, however, that in the reported instances of mutation the variations arose as the result of changes in the germ plasm. A mutation, therefore, has its origin from within and this origin has no very evident connection in any way with external conditions. If a case of an acquired character is shown to be inherited it is clear that the germ plasm of the organism must have been affected. The stimulus to change, therefore, in contrast with the cause of mutation, would have come from without. While the end result—the alteration of the germinal constitution—is the same in both cases, the method or cause by which it is brought about is different. From this point of view, the relative value in evolution of mutation and of hereditary acquired characters is open to various interpretations. I think that in the case of mutations it may soon be possible to demonstrate that some of the so-called examples of "mutations" are due to or are associated with irregularities of karyokinesis. It is not at all inconceivable that outside conditions producing ac-

quired characters may at the same time effect chromosome structure and behavior in the germ plasm. The cause for unusual activity on the part of the chromosomes in either case may, however, well be totally different.

Interesting as are the effects of natural influences on the germ plasm, of greatest importance is the problem whether or not modifications may be produced and controlled artificially. The fact that experimental research is tending to show the specificity of certain chemicals for various organisms or parts of organisms makes hopeful the outlook for finding a specific or many specifics that will act upon the mechanism of heredity or different parts of that mechanism. At present our investigations can only be empirical, trying this or that method more or less blindly. Considerable work in this field has already been done. Without entering into a full discussion of the numerous investigations, it may be said that alcohol, temperature, humidity, ether, zinc sulphate and radium have been used in attempts to alter the germinal constitution. Changes indeed have been produced in some cases but the effects have been generally physiological in nature either interfering with development or influencing color, length of hair, etc. In all cases where the offspring resembles the altered parents the results have been readily interpreted on the assumption that eggs or embryos are influenced at the same time as were the forms producing them. MacDougal reports having produced changes in one of the œnotheras through ovarial injections. Although it may be possible that he produced modifications, the plant he selected for experimentation was unfortunate since the natural variability of the œnotheras is in most cases great, and the gametic purity of his material was not clearly demonstrated.

In many ways plants offer the most favorable material for the study of experimental variation. Many parts of the plant are adapted to experimentation. The pollen may be subjected to treatment, the ovaries may be injected with chemicals or otherwise handled, the seeds offer themselves to various manipulations, many stages of growing plants are available for experimentation, a variety of experiments may be conducted and, if there are numerous branches, controls may be maintained on the same plant. Coupled with these advantages are possibilities of cultivating pure lines for many generations. It would be difficult indeed to find an animal about which all of the above statements could be made.

Two methods of inducing germinal variation seem practical. The first is the treatment of the germ cells. MacDougal's ovarial injections lie in this class. The other method is to attack the buds or growing points of the stems. I do not at present believe that exact results can be expected from ovarial injections since the ovules are generally so tightly packed that fluids can not readily circulate and it is impossible to know the quantity and the strength of the fluid that may reach the germ plasm. The treatment of pollen may offer greater possibilities unless unforeseen technical difficulties are encountered. By subjecting growing points to treatment the germ plasm may more readily be affected and the complete chromatin equipment may be placed under the influence of the materials used. If germinating seeds or seedlings are immersed in a given chemical solution we may have reason to expect that the cells of the growing points are probably in contact with the solution or some derivative of it. If the chromosomes of a growing point can be influenced it is possible that the organs that are developed from this point may be altered.

Two years ago, in connection with a program of study dealing in general with problems of development, I had the opportunity of making some experiments on seeds and seedlings of *Œnothera biennis* L. (the Dutch *biennis*) from a pedigreed line which had been inbred for at least eight generations. *Œnothera biennis* L. is one of the most stable species so far studied in this genus and its rare "mutations" are known from the research of Professor DeVries. Consequently it seemed justifiable to anticipate that any results obtained through experimental treatment may readily be recognized. To Dr. Bradley M. Davis I am indebted for suggesting *Œnothera biennis* as a favorable plant for study, for many pedigreed seeds and for the complete freedom of his garden. Without his aid in the study of the plants and without his advice I should have made little progress. In preliminary studies of this sort all the investigator can hope to accomplish is to determine certain solutions which produce suggestive effects. The results of my studies are here brought together in hope that they may be of some help to other workers.

As has been pointed out, studies of this character must at present be largely empirical. The problem is to find chemicals that will modify the structure of the germ plasm or bring about irregularities in the distribution of the chromosomes. Some of

the chemicals used are those frequently employed in fixing fluids; others were selected for various reasons.

As shown in the List of Experiments the seeds and seedlings were soaked for varying lengths of time in the solutions. The material was either thoroughly washed before being placed on moist filter paper in petri dishes to complete germination or it was placed on paper which had been moistened with the same solution as that in which the material had been soaked. Since the seeds of *Œnothera biennis* are about 96 per cent. viable, seed sterility was not an important factor in the results of the experiments. Dr. Davis's large cultures under normal conditions were used as controls. About one hundred seeds were used in each experiment.

List of Experiments

Fluids	Percentage	Seeds or Seedlings	Time in Solution	Percentage of Germination
Acetic acid	0.125	Seeds	4 days	25
	0.125	Seedlings	3 days	
	0.625	Seeds	29 days	40
Butyric acid	0.25	Seeds	34 days	40
	0.5	Seeds	34 days	0
	1.0	Seedlings	1 day	
	1.0	Seeds	5 days	0
Chloral hydrate	0.75	Seedlings	5 days	
	0.75	Seeds	34 days	50
	0.375	Seeds	24 days	0
	0.187	Seeds	24 days	50
Chromic acid	0.03	Seedlings	11 hours	
	0.015	Seeds	4 days	65
	0.015	Seeds	3 days	60
	0.015	Seedlings	21 days	
	0.015	Seeds	3 days	60
Ethyl alcohol	5.0	Seedlings	8 days	
	5.0	Seeds	27 days	0
	1.0	Seeds	18 days	40
	0.5	Seeds	3 days	60
	0.5	Seeds	24 days	80
	0.25	Seeds	24 days	A few germinations
Methyl alcohol	1.0	Seeds	18 days	Normal germination
Amylic alcohol	1.0	Seeds	18 days	0
Butylic alcohol	1.0	Seeds	18 days	0
Propylic alcohol	1.0	Seeds	18 days	0
Zinc sulphate	10.0	Seeds	18 days	0
	5.0	Seeds	18 days	A few germinations
Strychnine		Seeds	10 days	0
Pot. bromide and iodide		Seeds	10 days	0
Ferric alum	4.0	Seeds	10 days	0
	0.5	Seeds	14 days	19

Results

In the acetic-acid solutions mold grew vigorously and possibly interfered with the growing plants. The percentage of germination was low. In the young plants the cotyledons were rather more pointed than normal, although this modification was not marked. The leaves of young rosettes also appeared more narrow and pointed, but these peculiarities disappeared as the plants matured.

All the seeds and seedlings treated with butyric acid died.

Chloral hydrate produced no effect other than retarding the period of germination, reducing its percentage, and weakening the plants.

Chromic acid produced by far the most interesting results. In the various solutions used germination was prompt (about the usual three to four day period) but the percentage was materially lowered. The seedlings produced were vigorous in appearance, although the root system was in most cases stunted. There was a slight though not, I believe, significant modification of the cotyledons which were somewhat less pointed than in the type. Some of the seedlings were bright red and practically all had a reddish or pinkish tinge. Growth after planting was slow but all the plants finally developed normally.

Ethyl alcohol produced no modification of structure, although I believe that it will be worth while to continue this line of experimentation. In all cases where seeds were allowed to soak in ethyl alcohol the solution became thick and gelatinous from a substance extracted from the seeds. The percentage of germination was much reduced.

Methyl alcohol retarded germination but the resulting plants were fairly normal.

Amylic, butylic and propylic alcohols all inhibited germination in the strengths employed.

Germination was also inhibited by the solutions of zinc sulphate, strychnine, potassium bromide and iodide and by four per cent. ferric alum.

In general it may be said that the treatment of seeds and seedlings in the experiments has resulted, as in the experiments of others, in reducing the percentage of germination or in a general weakening of the plants rather than in specifically modifying the germinal constitution. The results from the experiments with chromic acid and possibly with chloral hydrate and ethyl alcohol suggest the desirability of further studies. In future

work the concentration of the agent and the length of treatment should be studied in greater detail.

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CONCERNING A MORPHOLOGICAL PREDICTION FROM DISTRIBUTIONAL DATA AND ITS SUBSEQUENT VERIFICATION[1]

ALL species of the genus *Salpa* are notable for their two alternating generations. One, known as the "solitary" generation, produces offspring by budding and the buds, when fully mature, constitute what is known as the "aggregate" generation, or, as I shall call them, *zooids*. Each zooid produces, sexually, one of the solitary generation, the embryo being nourished and carried within the body of the zooid until after it has begun to form zooids of the succeeding generation.

The relation between the two generations, however, is not simple; and as there seems to be a widespread misconception regarding this relation, it is well to correct it.

One sees the statement in much of the literature that the solitary generation is asexual, that the aggregate generation is sexual (hermaphroditic), and that the developing embryo of the solitary form is carried within the body of its mother. Such, however, is not the case; Brooks having clearly demonstrated in his classical volume "The Genus *Salpa*," that the solitary form is, in the most literal sense, a female while the zooid it produces by budding is a male. In brief, the curious sexual relationships are as follows:

The solitary form, or female, produces immature males by budding, within the body of each of which the mother tucks away one fully developed and ripened egg together with its follicle. Before fertilization the egg is suspended by means of a fertilizing duct, which opens into the cloaca, into one of the blood channels of the newly formed zooid. The spermatozoa which are drawn into the pharynx of the zooid with the sea-water, are swept past this opening by the contraction of the muscles in swimming, and some of them enter, one of which penetrates to the egg and fertilizes it. The embryo, at an early stage, pushes into the

[1] A paper read before the Western Society of Naturalists, Friday, April 6, 1917, at Stanford University.

cloaca, carrying its wall before it, thus becoming inclosed in an epithelial capsule. This capsule is soon cast off and, in its place, a placenta is formed which, communicating with the blood-channels of the zooid, nourishes the growing embryo until birth. While the embryo projects into the cloaca it is not, at first, exposed to the water, but is enclosed by an epithelial capsule and, after its disappearance, by an embryo sac resembling somewhat the amnion of vertebrates. Later, but still while very young, this sac is distended and finally broken by the growing embryo and from then until birth, the embryo is directly exposed to the water in the cloaca, being fastened to the zooid only by the placenta and a narrow band of ectoderm which connects the neck of the placenta to the walls of the cloaca. After the embryo has reached maturity and made its escape into the water, the testis matures and the zooid becomes a fully developed male. Virtually, then, the mother salpa gives rise, by budding, to its sons; and each son serves first as a depository for one of its mother's unfertilized eggs, then as a living incubator within which its mother's daughter is housed and reared, and lastly as a father. Such is, in brief, the life-cycle of the *Salpæ*.

In *Salpa democratica*, to which the distributional data to follow relate, and which is the tiniest of all the *Salpæ*, the mechanism of budding is as follows: Within the body of the solitary salpa, at its caudo-ventral extremity, lies a compact intestinal tract called the nucleus. Closely associated with it is an organ, "the proliferous stolon," which by segmenting distally gives rise to the zooids. The zooids are budded off rapidly and no evidence is at hand that the stolon ever exhausts its capacity to produce them. The zooids remain attached to each other and to the stolon in the form of a chain and, as segmentation advances, they become crowded and pushed along a spiral path encircling the nucleus. The stolon undergoes more or less regular periods of segmentation and rest so that the zooids are developed in sets or blocks, all individuals in a single block being of approximately the same size and in the same stage of development. Each block contains from forty to sixty-five zooids, and as there are from three to four blocks present when the distal end of the chain is ready to emerge from the test-cavity to the exterior, the stolon carries in the neighborhood of two hundred zooids at one time.

In other species the chain remains intact and attached to the stolon after, as well as before, the zooids are protruded into the

water, but no evidence of this relative to *S. democratica* is recorded in the literature.

During June and July, 1908 and 1909, thirty surface-net hauls were made in the vicinity of San Diego when the temperature of the water was between 15°.9 and 18°.3 C., and forty-six when it was between 18°.4 and 20°.8 C. Solitary forms were captured in greater numbers per unit volume of water filtered from the *warmer* water, and zooids from the *colder* water. But *one or more* of both solitary forms and zooids were captured from a larger per cent. of these unit volumes in the colder than in the warmer water. This is better shown by the following table:

TABLE SHOWING SURFACE DISTRIBUTION OF *S. democratica* RELATIVE TO WARM AND COLD WATER

Temperature in Centigrade	No. of Hauls	Solitary Forms		Zooids	
		Abund.[1]	Freq.[2]	Abund.[1]	Freq.[2]
15.9–18.3.....	30	67	92	529	92
18.4–20.8.....	46	160	73	127	80

These data show that, while zooids occurred in greatest numbers in the places they frequented the most as would be expected, solitary forms occurred in greatest numbers in the places they frequented the least, or, to state it differently, they were found most often where they occurred in smallest numbers. What does this apparent paradox mean?

Believing that these relations must have been consequent upon a freak result of chance or random sampling, and discovering that only 9 of the 30 cold-water hauls were made during the night (6 P.M.–6 A.M.) while 33 of the 46 warm-water hauls were made at night, the day hauls and night hauls were separated, and the data concerning each were retabulated with respect to the same two groups of temperatures. The results are given in the table on p. 575.

Be it noted that, although the magnitudes of the differences vary, the directions of the differences are exactly the same as revealed by the data in the above table, *i. e.*, solitary forms are most abundant and least frequent in the warmer water, while zooids are most abundant and most frequent in the colder water.

Still being in the position of an unbeliever, the data were re-

[1] Abundance or number of individuals per unit volume of water filtered.

[2] Frequency or per cent. of unit volumes containing at least one individual.

tabulated in every practicable manner: first, by eliminating all hauls except those made between 6 and 10 A.M.; second, by eliminating all except those made between 10 A.M. and 2 P.M.; third, by eliminating all except those made between 6 and 10 P.M.; fourth, by eliminating all except those made during June, 1909, and fifth, by eliminating all except those made between June 12 and 30, 1909, and within a distance of approximately half a mile of each other. But, in every case without exception solitary forms were most abundant and least frequent in the warmer water, while zooids were most abundant and most frequent in the colder water. Furthermore, the frequency of solitary forms was found to be usually identical with that of zooids. Finally, the probability of such an identical series of relations being due to the effects of random sampling was computed, and the odds against it were found to be more than 1,600 to 1. Obviously, then, the apparent paradox has a significance. What is it?

DAYLIGHT HAULS (6 A.M.–6 P.M.)

	Hauls	Solitary Forms		Zooids	
		Abund.	Freq.	Abund.	Freq.
Cold water	21	73	96	710	96
Warm water	13	281	92	158	86

NIGHT HAULS (6 P.M.–6 A.M.)

Cold water	9	58	81	220	81
Warm water	33	135	63	118	79

Let a solitary form be symbolized by a cork, a zooid by an iron weight, warm water by the surface of the ocean, and cold water by the bottom of the ocean. Flotation is then analogous to accumulation in warm water, and sinking to accumulation in cold water. The problem may then be restated as follows:

Since corks float and iron weights sink, what is the relation between them that necessitates taking some corks from the bottom of the ocean whenever a number of iron weights are taken therefrom, and that necessitates taking some iron weights from the surface whenever a number of corks are taken therefrom?

Stated in this symbolical language, it is obvious that the only feasible answer is that at the time the corks and iron weights were removed from the ocean, they were tied together. Now, if,

by experiment, we find that one cork will barely float say six iron weights, corks with more than this number attached would sink, while those with less attached would float. Moreover, if those corks with more than six weights attached usually outnumbered those with less, while occasionally those with less far outnumbered those with more, then both corks and iron weights would be so distributed with respect to surface and bottom that, while corks in the long run would be obtained in greater numbers from the surface than from the bottom, at least one would be obtained in a greater percentage of bottom than of surface hauls. In other words corks would be most abundant and least frequent on the surface, while iron weights would be most abundant and most frequent on the bottom—the exact parallel of the distribution of the two generations of *S. democratica*.

The conclusion, therefore, seems unescapable that the zooids, after being pushed to the exterior on the proliferating stolon of the solitary salpa, remain attached to each other and to the stolon in the form of a protruding chain.

More positive proof being mandatory, each of the seventy-six hauls were carefully examined but, although certain statistical facts pointed toward the existence of protruding chains, no two zooids were found attached together. But, being convinced against my own prejudice, that such chains were encountered and broken up during the processes of towing and washing the net, hauls of brief duration were made, and in some of them several fragments of chains were discovered. Comparison of the size of these zooids with that of the largest ones found within the test-cavity of the solitary salpa proves them to have remained attached to their progenitor for a considerable period, probably until at least one entire block of zooids had reached the exterior.

The distributional data carry many other significant implications which can not be discussed in this limited time. Suffice it to say that a complete report is now nearly ready for the press and will be published in the Zoological series of the University of California under the title "Differentials in Behavior of the Two Generations of *Salpa democratica* Relative to the Temperature of the Sea."

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THE MUTATION THEORY AND THE SPECIES-CONCEPT[1]

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IN the early days of natural history, when the conceptions of special creation held sway, it was supposed that any one could determine species who was capable of observing the differences between existing forms. Linnæus crystallized this sentiment into the dictum that there are as many species as were created in the beginning, implying that any one with sufficient powers of discrimination could determine exactly how many species there were in each group. But with the introduction of the theory of evolution, species came to be viewed more and more as dynamic entities, and questions of origin have entered progressively into the species-concept. The latter has grown continually more complex, and yet Darwin's anticipation that systematists would cease to discuss how many Rubi there were in Britain or how many Crataegi in North America, has not been realized.

On the contrary, with this increase in the complexity of the conception of species, the extreme views as to what constitutes a species have become more and more divergent, until the "lumpers" and "splitters" among systematists usually differ radically in their interpretation of the species in a given genus. This diversity of opinion among systematists has been partly a direct result of our increasing knowledge of the complexity of species, de-

[1] Presented at the Pacific Coast meeting of the American Association for the Advancement of Science at San Diego, August, 1916.

rived from studies of variation and geographical distribution and from the experimental study of evolution.

FIG. 1

Key to Map

1. ***Otus asio asio*** (Linn.) Ridgway (=*floridanus*).
2. ***Otus asio naevius*** (Gmel.) Ridgway.
3. ***mccallii*** (Cassin) Ridgway.
4. ***hasbroucki*** Ridgway.
5. ***aikeni*** (Brewster) Ridgway.
6. ***maxwelliae*** (Ridg.) Ridg.
7. ***macfarlanei*** (Brewster) Ridgway.
8. ***kennicottii*** (Elliot) Ridgway.
9. ***brewsteri*** Ridgway.
10. ***bendirei*** (Brewster) Ridgway.
10a. ***quercinus*** Grinnell.
11. ***cineraceus*** (Ridg.) Ridg.
12. ***gilmani*** Swarth.
13. ***xantusi*** (Brewster) Ridg.
14. ***Otus trichopsis*** (Wagler) Ridg.
15. ***Otus vinaceus*** (Brewster) Ridg.

If we look for a moment first at the complexities which have been added to the original simple concept of species, we find all grades and kinds of difference within the species itself, such as subspecies, varieties, forms and races, ending finally in the differences between single individuals. Some of these conceptions as ordinarily used are also related to geographical distribution. To them

must be added from experimental work the conceptions of mutants, Mendelian units, phænotypes and genotypes, and pure lines differing only in the position of their modal condition and requiring statistical analysis for their demonstration.

With such an array of apparently (though not really) conflicting concepts before him, it is small wonder that the systematist is inclined to cast them all aside and describe his species according to his own ideas of what they are and how they have originated. Nevertheless, for him as for the experimentalist the question What is a species? is more or less colored, if not determined by the question What is its origin? How did it appear? It might be said that the systematist should pay no attention to origins at all, merely describing what he sees. Some systematists doubtless adopt this plan. But obviously, for an understanding of the characters and relationships of species all possible facts and conceptions bearing on their origin should be considered, and in this way systematics may ultimately hope to become something more than a purely descriptive science.

If we examine the ideas which form the background of thought of the systematist in his work of constructing species, we find almost invariably that they are based entirely upon the Darwinian conception of natural selection by the gradual accumulation of slight individual differences. We are then concerned to ask, Is the systematist justified in assuming that all specific and varietal differences have originated in one way? I can find no reason in logic or philosophy why this should be the case; and for ourselves, we believe there is no single method of species formation, but we think the conclusion will ultimately be reached that the methods of species formation are multiform, though certain of them are doubtless more widespread and important than others. Among the more important factors in speciation which we wish to consider here should therefore be mentioned (1) local adaptation of races through natural selection or by direct response

to environment, (2) mutations occurring more or less independently of the environment and not necessarily of adaptive value, (3) orthogenesis, whatever that may imply. These are by no means mutually exclusive, and we can see no reason why all, and others as well which we have not time to consider, should not have been at work producing the result we call organic evolution.

It is not difficult to find, particularly among birds and mammals, instances of specific variation giving rise to local geographic races which are apparently the result of response on the part of the organism to local conditions. Again, it is easy, particularly among plants, to find varieties, species and even genera which have arisen apparently through sudden mutations and without anything in the nature of adaptational response. Finally, paleontology teems with apparent cases of orthogenetic phylogeny which are not at present clearly explainable in terms of natural selection or mutations. Before examining concrete cases which come under each of these categories let us diverge for a moment to consider the effect which natural selection as a theory has had upon biological conceptions.

We see at once that the philosophical conception of continuity took an extraordinary hold on the minds of biologists. Largely as a result of the great influence of Darwin, towards the end of the nineteenth century continuity in the origin of species became almost a fetish, and all efforts were directed to showing how every character whatsoever might have originated through the selection of a series of gradually intergrading infinitesimal steps. Yet it is more than doubtful if Darwin himself would ever have been led into such an extreme position. Biological philosophy has thus been ridden with the conception that if a character could be shown to have arisen in a gradual, piecemeal fashion its origin was thereby explained or accounted for, even though natural selection could not be shown to have operated in its development. On the other hand, the appearance of a character sud-

denly, at one step, was considered practically equivalent to its creation by a miracle, and the type of argument involving this view is still not infrequently leveled against the mutation theory.

But where lies the necessity for assuming that either continuity or discontinuity is universal? Surely the matter is one to be determined by direct observation, and not by *a priori* argument. The continuity concept of origins appears not to have influenced other sciences to the extent it has biology. True, Lyell first introduced it by developing the doctrine of uniformitarianism in geology. Nevertheless the geologist has continued to deal with large and relatively catastrophic effects occurring at irregular intervals, such as landslides, floods, earthquakes and volcanic eruptions. The phenomena of geological history are then continuous only in a limited sense.

Similarly, no chemist supposes it necessary to think that, for example, carbon and silicon were gradually differentiated from some previous substance which possessed certain qualities of both. On the contrary he sees his atoms built of definite units, the electrons, combined in various ways and numbers to give a variety of products, the elements, which are for the most part stable from the first. Hence, while perhaps little can be gained for the biologist by reasoning from analogy with other sciences, yet we at least realize that concepts of discontinuity are quite as widespread in science at large as are those of continuity, and that the origin of a character is not explained merely by breaking it up into infinitesimal steps through which it may not have passed at all.

Let us consider now some concrete instances. And here we shall select chiefly cases of discontinuity, since we are considering especially the bearing of the mutation theory on the conception of species. In examining species and genera of plants and animals, we find very often, particularly in plants, characters which almost certainly had a discontinuous origin. Perhaps the majority of generic

characters in higher plants have originated in this way. Such morphological generic characters are found in numbers wherever one turns. They indicate a great variety of marked changes, in addition to those involving alteration in number of parts; they most often concern the flower structure, on which generic differences usually depend; and in many cases at least they can not reasonably be supposed to be of any special value to the plant.

If we turn to the lily family and compare the well-known North American genera, *Smilacina* and *Maianthemum*, we find the following differences:

Smilacina	*Maianthemum*
Perianth segments 6	Perianth segments 4
Stamens 6	Stamens 4
Ovary 3-celled	Ovary 2-celled
Style short and thick	Style about as long as the ovary
Stigma 3-grooved or 3-lobed	Stigma 2-lobed or 2-cleft
Leaves oblong or lanceolate	Leaves usually cordate at base

These generic differences are almost entirely in the number of parts in the flower. Otherwise, in foliage and habit *Maianthemum* might be considered a reduced boreal subgenus of *Smilacina*. Can it be doubted that *Maianthemum* originated from the ancestors of certain species of *Smilacina* through a mutation, in which the flowers changed suddenly from the hexamerous to the tetramerous condition? All these changes in flower-parts would then have occurred at one stroke. It can not well be imagined that they passed through a series of gradual transition stages which have since been lost. When one remembers the almost universal occurrence of 3-parted flowers in Monocotyledons, this change becomes all the more striking. The whole order Cruciferæ, among Dicotyledons, must have originated in the same way, through a sudden change from pentamery to tetramery.

If we examine the species of *Maianthemum* and their varieties we find evidence that similar processes of discontinuous variation are going on at the present time. The genus contains three species, *M. canadense* in Amer-

ica, *M. bifolium* in Europe and *M. dilatatum* in western America and northwestern Asia. *M. canadense* differs from *M. bifolium* chiefly in leaf-shape and in being typically glabrous. The pubescence probably was lost at one stroke, just as numerous glabrous varieties arise. Another step is sufficient to account for the alteration in leaf-form, so that two steps are ample for the transition from one species to the other. *M. dilatatum* resembles *M. bifolium* except for its relatively gigantic size and the fact that it is glabrous like *M. canadense*. Again two definite steps are sufficient to account for its origin.

Turning now to the geographic variations of *M. canadense* particularly as regards pubescence, a detailed study shows that over the greater part of its extensive range it is absolutely glabrous, but that pubescence has appeared especially in two localized parts of its range. A heavily hirsute variety *interius* Fernald, occurs in the Black Hills of South Dakota, an exceedingly arid region on or near the western extremity of the range of the species. This variety is apparently restricted in its distribution to the arid portion of western South Dakota, and the most reasonable interpretation appears to be that it has originated here through a marked variation, and has thus enabled the species to extend its range into this arid region. Further east, chiefly in Minnesota and Wisconsin, a semipubescent form occurs, and this may be the form from which the much more marked hirsute variety arose. The evidence, when closely examined, favors then a discontinuous rather than a continuous manner of origin of this heavily pubescent condition. The condition itself is nevertheless an adaptation, enabling the plant to survive in extreme conditions of aridity.

The monotypic genus *Kruhsea* is related to *Streptopus* in much the same way that *Maianthemum* is related to *Smilacina*. It was originally described in the "Flora Rossica" by Ledebour as *Smilacina streptopoides* from eastern Siberia. His name indicates his idea of its relationships. Baker afterwards, from plants without flowers

collected in Oregon, described what has proved to be the same form under the provisional name *Streptopus brevipes*. *Kruhsea streptopoides* then, although it agrees with *Streptopus* in foliage and in fruit and seed characters, differs remarkably in its flowers. They are very small, the perianth nearly rotate, dark purple; the stamens altered; and in the absence of a style the discoid stigma rests directly on the ovary. It is possible that connecting forms between *Kruhsea* and *Streptopus* may yet be found in Siberia, but at any rate the differences between these two genera can not be reasonably supposed to have arisen through natural selection. *Kruhsea* appears to have originated through a few definite germinal changes and to have since been perpetuated by heredity.

Another pair of genera which is of much interest in this connection is *Platystemon* and *Platystigma*, two Californian genera of the Papaveraceæ. Both occur abundantly as spring flowers, occupying similar habitats. Their main differences are as follows:

Platystemon	*Platystigma*
Stamens numerous	Stamens 6–12
Filaments broad and flattened	Filaments narrower, flattened or filiform
Carpels 6–20, forming a compound ovary, which in fruit breaks up by constrictions into 1-seeded joints	Carpels 3, forming a 1-celled, 3-valved or terete ovary which in fruit forms a 3-valved dehiscent capsule

These genera are almost exactly alike in habit, foliage, pubescence, color of flowers and general form of the stamens. They differ chiefly in the pistils, and these differences only become conspicuous as the seed capsules mature. *Platystemon* has acquired numerous carpels which are connivent or coherent in a circle. In developing, the carpels separate and their free margins cohere with each other. Each carpel then becomes torulose by constrictions between the seeds. How shall we account for the origin of such a condition except through a marked

variation, which is perpetuated by heredity and not because the plant has any advantage or disadvantage in life compared with *Platystigma*. Only one species of *Platystemon* (*P. californicus* Benth.) and two of *Platystigma* have been generally recognized, although Greene[2] has described some 50 species based on minor differences.

Another significant difference between *Platystemon* and *Platystigma* is in the variations of the petals. In *Platystemon* the number varies from 6 to 10 or more, and all the petals of a flower or a plant vary together in color from dark yellow through light yellow to white. In *Platystigma*, on the other hand, the number of petals appears to be uniformly six, and the outer three vary in color independently of the (alternate) inner three. Thus in *Platystigma lineare* Benth. (which Greene calls *Hesperomecon pulchellum*) the outer petals may be dark yellow, or with a more or less extensive wedge-shaped dark yellow mark at the tip, while the inner petals are light yellow or white.[3]

The peculiarity in the carpels of *Platystemon* acquires added interest from the fact that, as Lindley pointed out,[4] it is by no means unique, but contraction of the sides of the carpels, forming a torulose structure, has occurred equally and must have originated independently in *Hypecoum* of the Papaveraceæ, in such genera as the radishes among Cruciferæ, in *Ornithopus* among the Leguminosæ, and in other families. We may look upon this condition as apparently the result of parallel mutation in different families, independent of utility; and countless other cases of a similar kind occur among higher plants.

From the few instances I have cited, which could be added to indefinitely, and from the abundant evidence of marked variations which we have from experiment, the

[2] Greene, E. L., 1903, "*Platystemon* and its Allies," *Pittonia*, 5: 139–194.

[3] A figure of *P. lineare* in *Bot. Reg.*, T. 1954 (1837), from the Russian River, Cal., shows the petals alternately yellow and white.

Another interesting point, to which Mrs. K. Brandegee has directed my attention, is the abundant occurrence of tiny plants bearing a single minute flower, intermingled with the larger plants.

[4] *Bot. Reg.*, T. 1679 (1834).

conclusion seems clear that many marked morphological characters in plants have arisen independently of function and without the aid of natural selection. This conclusion is all the more probable because form is so much more loosely tied to function in plants than in animals. In many plants it makes little or no difference what is the shape of the leaf so far as its chlorophyllian function is concerned, nor what is the shape of the anthers so long as they produce pollen.

Another matter, which I have touched upon elsewhere,[5] is the geographic relationships of the most closely related species of plants. It appears that Jordan's well-known law that the most nearly related species occupy adjacent areas, although widely applicable especially to the subspecies of mammals and birds, is by no means so generally true in regard to plants. But we shall come to this point again.

Referring now to animals, the North American screech owls afford an interesting case in which two kinds of variability can be clearly contrasted as regards their geographic relationships. These two types of variations are (1) those in which apparently continuous or nearly continuous variations occur progressively over certain geographical areas, with no two forms occupying the same area, and (2) those in which two or more sharply marked forms occupy the same area.

The accompanying map, compiled largely from Ridgway's data,[6] shows the distribution of the various sub-

[5] Gates, R. R., 1916, "On Pairs of Species," *Bot. Gazette*, 61: 177–212. Figs. 12.

[6] Ridgway, Robert, 1914, "The Birds of North and Middle America," *Bull. U. S. Nat. Mus.*, No. 50, Part VI, pp. 882, pls. 36.

Ridgway says (p. 683): "In the main, geographic variations [in *Otus*] are more or less marked and constant; but occasionally specimens occur in a given area which are with difficulty, if at all, distinguishable from the form inhabiting another—sometimes distant—geographic area." He further comments on the fact that, while *O. choliba* in South America is remarkably uniform over a vast area, *O. asio* shows great change of coloration within relatively short distances, indicating an organization sensitive to slight changes in the physical environment.

species of *Otus Asio* Stephens (formerly known as *Megascops asio* Kaup) over the North American continent. While such a map is only approximately accurate, it shows that in general only one subspecies occupies a given geographic area.[7] There are, however, certain exceptions. Thus in Central Colorado *Otus asio aikeni* and *Otus asio maxwelliæ* both occur, the former finding here its northern limit from Texas and Mexico, the latter the southern limit of its range from Montana. It is stated, however,[8] that they occur in Colorado chiefly at different altitudes, *maxwelliæ* up to 6,000 ft. and *aikeni* from 5,000 to 9,000 ft. This is the reverse of what might be expected, since *aikeni* is the more southern form. But Mr. Aiken states[9] that at Colorado Springs *maxwelliæ* occurs only in winter and *aikeni* only in summer, indicating a slight migration. Again, *gilmani* and *cineraceus*—the latter somewhat darker with coarser pencilings and averaging slightly larger in size—both occur in southwestern Arizona, but, according to Swarth,[10] although both birds may occasionally be taken in the same locality, this is only in winter when *cineraceus* comes down from the higher altitudes to the different life zone of the hot Lower Sonoran valleys occupied by *gilmani*.

The differences between these various subspecies are chiefly in density of coloration and in size.[11] Thus *Otus asio nævius* is larger than *Otus asio asio* and is also lighter in coloration, with more white on the under parts. The subspecies *mccallii* in Texas and northern Mexico is intermediate between these in size, but is paler than either

[7] It may be pointed out that there is sometimes discernible a tendency for systematists to call a form a subspecies or a species according to whether or not it is the only form in a given area, thus making the geographical relations of the form their criterion, rather than the degree of its distinctness.

[8] Cooke, W. W., 1897, "The Birds of Colorado," Bull. No. 37, Agric. Expt. Sta., Fort Collins, Colo., p. 78.

[9] Cooke, W. W., 1898, "Further Notes on the Birds of Colorado," Bull. No. 44, Agric. Expt. Station, Fort Collins, Colo., p. 160.

[10] Swarth, H. S., 1916, "The Sahuaro Screech Owl as a Recognizable Race," *Condor*, 18: 163–165.

[11] I am indebted to Dr. Grinnell for permission to examine series of specimens in the Museum of Vertebrate Zoology of the University of California.

and more coarsely mottled. *Hasbroucki*, very limited in known range (see map), is decidedly larger and darker than *mccallii*, with much less buffy gray above and broader transverse bars. *Maxwelliæ*, another northern form, is decidedly larger but paler than *aikeni*. It is the palest of all in color, with more extensive pure white than even *nævius*. West of *maxwelliæ*, in Washington and Oregon, is *macfarlanei*, which is larger and very much darker, almost agreeing in coloration with *bendirei* of California.

The Pacific coast forms comprise an interesting series running down the coast, beginning with *kennicottii*, which occurs from Sitka through British Columbia to the southern border of Washington State. It is very large like *macfarlanci*, but much darker, and browner rather than gray. The remaining subspecies extending down the coast region and into the desert become progressively paler and smaller. Thus *brewsteri* in Oregon is smaller and less brownish than *kennicottii*. In California occurs *bendirei* which is lighter again and smaller. Grinnell[12] has segregated from *bendirei* in the more arid region of southern California another form under the subspecific name *quercinus*, considered to be paler dorsally and with less or no ferruginous markings around the head. But I confess that this difference, if it exists as a constant distinction, is too fine for me to appreciate. On the contrary, specimens of *bendirei* from Palo Alto appeared to me somewhat lighter on the breast than a series from Pasadena. Whether or not this very close form is distinguishable from *bendirei*, the next in the series are *cineraceus*, *gilmani* and *xantusi*, becoming progressively lighter with finer vermiculations, the two former in southern Arizona and *xantusi* confined to the tip of the peninsula of Lower California, smaller and with the toes less feathered.

Thus the subspecies appear to be arranged progressively in passing from one geographic area to another, and there is little overlapping. But this conception of

[12] Grinnell, J., 1915, "A New Subspecies of Screech Owl from California," *Auk*, 32: 59–60.

gradual and progressive change can be overdone when it is attempted to correlate the alterations observed with climatic or other environmental features. Thus the progressive lightening in color from *kennicottii* to *xantusi*, first by lightening and restriction of the brown until it practically all disappears and then by paling and diminution of the gray, is believed to be associated with the decreasing moisture in the northern part of the range and the increasing aridity in the south. There are of course many well-known cases of paler races of birds and mammals occupying desert areas. Yet it is not clear that the coastal region of Oregon, where the less dense brown *brewsteri* occurs, is any less humid than the corresponding part of Washington where *kennicotti* is found. Similarly *xantusi* on the peninsula of Lower California can not be supposed to exist in a drier habitat than *gilmani* or *cineraceus*. Of course in none of these cases is it known just what feature in the environment acts as the critical factor nor how the race responds to it. The experimental studies of Tower[13] and others show that a race may respond in the same way (*i. e.*, by showing the same variations) to different environmental stimuli or in different ways to the same stimulus. But studies of this character are still too few to furnish a basis for interpreting these reactions on the part of species of the higher animals. The experiments being carried on by Sumner[14] with the white-footed mouse, *Peromyscus maniculatus* may be expected to throw further light on this important question of the origin of local subspecies.

Again, it is not certain that such races as *kennicottii*, *brewsteri* and *bendirei* form an absolutely graded series with all intermediates. On the contrary there appears to be some evidence that although their boundaries are contiguous there are definite though small steps from one to

[13] Tower, W. L., 1906, "An Investigation of Evolution in Chrysomelid Beetles of the Genus *Leptinotarsa*," Carnegie Inst. Publ. 48, pp. 320, figs. 31, pls. 30.

[14] Sumner, F. B., 1915, "Genetic Studies of Several Geographic Races of California Deer-mice," AM. NAT., 49: 688–701, with map.

the other. This may conceivably be explained through the principle of invasion and reinvasion. Grinnell,[15] who is doing so much towards a detailed knowledge of the Pacific coast fauna, has considered this principle and also the part played by barriers in the development of geographic subspecies or races, in connection with the discussion of many specific cases of distribution in birds and mammals. Walter P. Taylor,[16] in a recent study of the western beavers, concludes in agreement with others, that migration, geographic isolation with adaptation to local ecological niches, and final reinvasion of earlier-occupied localities, will account for the origin and present distribution of geographic subspecies such as we have been considering. This explanation seems as likely as any other at the present time, but it is beyond the purpose of the present paper to discuss these aspects of speciation in birds and mammals. The intention is rather to show that the problems involved are entirely different from those concerned with another type of variability to be mentioned in a moment. It may be pointed out, however, that although the theory of reinvasion as developed involves the conception of races isolated in certain geographic areas becoming gradually modified through environmental stress and fixed before the reinvasion takes place; that there is at the present time no definite evidence that fixation actually takes place gradually, in this way or in any other way. If intermediates between the various geographic subspecies do not occur, this may be because definite though small steps in variation are taken from one race to the other, which would do away with the necessity for assuming a long period of isolation during which the gradual development and fixation of the race

[15] Grinnell, Joseph, 1914, "An Account of the Mammals and Birds of the Lower Colorado Valley, with Especial Reference to the Distributional Problems Presented." *Univ. Calif. Publ. Zool.*, 12: 51–294, 9 figs., pls. 3–13, and other papers.

[16] Taylor, Walter P., 1916, "The Status of the Beavers of Western North America, with a Consideration of the Factors in Their Speciation," *Univ. Calif. Publ. Zool.*, 12: 413–495.

occurred. However, the process does appear to be gradual at least in comparison with the other type of variability, which is fundamentally different in its geographic relations.

The second type of variability in *Otus asio* to which I have reference, consists in the occurrence of gray and reddish or rufous phases of coloration in the same area of distribution. Thus all the eastern subspecies, *asio, nævius, mccallii* and *hasbroucki,* produce both gray and red birds. These phases are sharply marked, and intermediates rarely occur. Hasbrouck[17] attempted an explanation of this dichromatic condition, but some of his conclusions were justly criticized by Allen.[18] The gray phase occurs more commonly in Florida and in the northern part of the range of *nævius,*[19] while the red phase occurs commonly in the Central Atlantic states, perhaps to the exclusion of the gray in some localities. The red phase is unknown in the western forms of *Otus asio.* Nevertheless grayish and rufescent phases of the small *O. flammeolus,* which is found in the mountains of western America from British Columbia to Mexico, occur in this region. The red phase is found also in *O. trichopsis* (see map). Similarly, brown and rufous phases are found in *O. choliba* which extends over a large part of South America, and also in the Central and South American species *O. cassini, O. guatamalæ, O. barbarus* and *O. vermiculatus.*

Owls belonging to other genera also exhibit two phases. For example *Bubo virginianus* (*Asio magellanicus*),[20] the single species of *Bubo* occurring in all North and South America, with many geographic varieties, shows dichromatism in various parts of its range. The same is apparently true of various Old World owls.

[17] Hasbrouck, E. M., 1893, "Evolution and Dichromatism in the Genus *Megascops,*" AMER. NAT., 27: 521–533, 638–649, 4 maps.

[18] A(llen), J. A., 1893, *Auk,* 10: 347–351.

[19] Oberholser, H. C., 1904, "A Revision of the American Great Horned Owls," *Proc. U. S. Nat. Mus.,* 27: 177–192.

[20] The red phase is stated by Allen to be rare in Maine.

Dichromatism is then, both geographically and systematically, a widespread phenomenon in owls. The red phase appears to be quite independent of geographic locality in its origin. Hasbrouck attempted to show with regard to *Otus asio* that the red phase had arisen gradually from the gray, which it was slowly supplanting in certain areas. He believed that the grays inhabited regions of greater humidity (Florida, northern range of *nævius*) and the reds the drier interior, yet grays occur in Florida and reds are found, though uncommonly, in Maine. He also reported reds as occurring exclusively in the relatively humid Mississippi valley. But, as Allen pointed out, any such correlation with climatic or environmental factors hopelessly breaks down because both types are found indiscriminately over at least the greater part of the eastern range. All writers agree that the two types of plumage are independent of age, sex or season, and that in many localities at least both occur together and freely interbreed. Hasbrouck states, however, that gray males far outnumber red males, while red females outnumber gray females 4:1. Confirmation of this point is to be desired, as it suggests sex-linkage of the red condition. It is further stated that on the continent of Europe the red owls are said always to be females and the grays males.

All young birds of *Otus asio* are gray in the down, the red first appearing in the feathers. Observations go to show that red birds mated with red may give (1) all red offspring, (2) all gray, or (3) both red and gray. When one parent is red and the other gray, the same three results may follow. Further, Hasbrouck claims that gray $\times$ gray gives always only gray young. This is probably true, but since the result is based on observation of only six matings of this kind in regions where reds occur, it is much to be desired that further observations on this point should be recorded.

The obvious hypothesis to explain these facts is that the red phase appeared as a mutation from the gray, and

that it is inherited as a simple Mendelian dominant character. The results of the various matings between red and gray would then be as stated above, according to whether the red parent were homozygous or heterozygous, but the offspring from red × red should seldom be all gray, since this would be only a chance result when both parents were heterozygous. It is not impossible, however, since the screech owls usually have only three or four young in a nest, or sometimes only two.

Since the red phase occurs in various species as well as subspecies it is not improbable that it has originated through independent variations in different species. In any case the geographic ranges of the red phases show that, having appeared as variations, they are inherited without any conspicuous advantage or disadvantage in competition with the gray. The present frequency of the reds in certain areas and their infrequency in others may be merely an indication of the localities where the original mutations took place, and from which as centers they have gradually spread.

Although the western forms have no red phase, yet *Otus asio kennicottii* exhibits in addition to its usual tawny-brown phase a relatively rare gray phase. This fact is indeed an argument favoring the assumption that the brown phase of *Kennicottii* also arose at one step and has since nearly supplanted the original gray form.

If now we compare the two types of variability that I have described in *Otus asio,* we find them sharply contrasted in several respects: (1) the former is clearly related to geographical distribution, a single race occurring in each locality: the latter has no such relation, but two forms may occur interchangeably in the same place; (2) the former is essentially continuous as a form of variation, the latter markedly discontinuous; (3) the former appears to be related to environmental (climatic) conditions, the latter apparently bears no such relation. As regards their evolutionary significance, there can be little doubt that the former or apparently continuous type of

variations is more important in this case, for they appear to have given rise to the geographic subspecies now recognized, and, moreover, the specific differences in the genus are merely an exaggeration or intensification of the kinds of difference shown by these subspecies. It seems evident, then, that the differentiation which has gone on in the evolution of the genus *Otus* is for the most part of the kind exemplified by the small differences now existing between geographic races or subspecies occupying different areas.

The same thing is true of many other birds and mammals, but this condition is by no means universal even in these groups of animals. On the contrary, it is not difficult to find instances in which the discontinuous type of variation, independent of environment or function, has been the main factor in speciation. I will merely mention the case of the North American flickers, *Colaptes auratus* and *C. cafer,* set forth by Bateson,[21] since the latter is a Californian bird. These species differ remarkably in their color markings, the most conspicuous differences being (1) yellow or red quills, (2) a black or a red malar strip in the males, (3) the presence or absence of a scarlet nuchal crescent in males and females. *C. auratus* possesses the first of each pair of characters and *C. cafer* the second. *C. auratus* extends from Alaska diagonally across Canada and the United States to Texas and eastward to the Atlantic, while *C. cafer* occurs in its pure form from Oregon through Utah, California and Arizona into Mexico. Each possesses 3 or 4 geographical subspecies. Where the ranges of the species overlap over a large area a mixed population of forms occurs which is usually interpreted as a series of complex hybrids, but this will bear further study. It is clear, however, as Bateson points out, that the differences in range of the species can not be associated with any constant environmental difference in the habitats, and that the species can not have differentiated from this mixed population of inter-

[21] Bateson, W., 1913, "Problems of Genetics," Yale Univ. Press, p. 146.

mediate forms. However these species originated, they can not be reasonably supposed to have developed through gradual adaptation, but the color differences probably play no more part in the economy of the species than is the case with the red and gray phases of the screech owls. Something in the germinal organization of *Colaptes* doubtless determines the definiteness of its color patterns, and it is probable that each element of the pattern was changed by a marked step rather than through a series of gradual stages. This view is strengthened by the fact that a third species, *C. chrysoides* in Lower California, is essentially a *cafer* with yellow instead of red quills.

Thus even in birds our second type of variation, non-adaptational and not related to local conditions, is apparently an important factor in speciation, although in *Colaptes* too geographical races occur as well. In distribution also these species do not follow the rule for geographic subspecies, for they overlap over large areas. The fact that each species has its own geographic subspecies shows that the origin of these species antedates the development of their geographic varieties.

I have endeavored to show that in plant and animal species there are two distinct types of variability, having different geographical relations. The one is discontinuous, independent of environmental or functional influence, and has given rise to many specific and generic characters, notably in plants but also in higher animals. The other is continuous and apparently represents the results of the stress of the environment on the species in its dispersal, leading to the gradual differentiation of local races or subspecies whose peculiarities are ultimately intensified and fixed. The latter type of speciation is notably exemplified in birds and mammals, organisms in which, unlike plants, the individuals can migrate from place to place and so substitute for a stress resulting from overpopulation an environmental stress caused by a new set of climatic or physiographic conditions.

FURTHER OBSERVATIONS ON THE EFFECTS OF ALCOHOL ON WHITE MICE[1]

L. B. NICE

In a former paper ('11) it was found that white mice were not markedly affected when given alcohol in their food. Since this paper appeared Stockard ('12, '13, '16) has brought forth some striking and conclusive results demonstrating that guinea pigs are very sensitive to alcohol and decidedly injured by it. He administered the alcohol to his animals through the lungs by placing them in a tank containing alcohol at the bottom so that they had to inhale the fumes. His work raised the question as to whether similar results might be obtained with white mice by using the same method. Therefore it was decided to repeat my experiments, using the inhalation method.

For these experiments white mice eleven weeks old were obtained. They were all from one strain inbred to the fourth generation, two entirely distinct strains having been united to form this strain. They were divided into four lines, viz., a control line, a double alcohol line, that is, both males and females were subjected to alcohol, a female alcohol line and a male alcohol line. There were three cages in each line, each cage contained two females and one male, thus making six females and three males in each line. Two cages were made up of second generation alcoholized mice; both males were from the male alcohol line, two females from the same line and one female from the female alcohol line. The same cages were used as in my former experiments. They were made of 8-mesh wire and were 6 inches wide, 6 inches deep and 12 inches long.

The mice were kept in a laboratory room heated by steam. It was attempted to keep the room at a uniform temperature, but fluctuations occurred.

[1] From the Laboratory of Physiology in the University of Oklahoma. For a review of the literature see Nice ('11 and '12). Also Stockard ('12, '13 and '16).

All the animals were fed the same food, consisting of wheat and kaffir corn with bread and milk once a day.

Every day except Sunday the double alcohol line, parents and young, the males of the male alcohol line, the females and young of the female alcohol line and the second generation of alcohol mice with their young were placed in a galvanized tank 26 inches long, 20 inches wide and 14 inches deep. Alcohol had been poured on to cotton which was placed under a wire mesh situated about 2 inches from the bottom of the tank, so the mice had to breathe the fumes. The mice were kept in the tank each day until they became intoxicated, as shown by their staggering gait or inability to stand up. At the beginning of the experiment the time necessary to intoxicate them was about one hour. Later they would often be kept in the tank for two hours. This shows that the mice acquired a tolerance for alcohol. This tolerance was shown after they had been treated about a month. To make sure that the mice were being heavily alcoholized, a few times they were left in the tank so long that they would not recover from the effects for three or more hours, and in some cases they did not recover from the intoxication, but died.

At the beginning of the experiment when the mice were placed in the alcohol tank they would sneeze, their eyes water and they would rush about in their cages, showing great uneasiness. Later they ceased to be so much disturbed, yet during the course of the experiment there was no indication that the mice liked the alcohol fumes.

The Weight of the Adult Mice

The adult mice were weighed at the beginning of the experiment and once each month thereafter, to get an indication as to their health. Since they were nearly the same age and closely related, their average weights would be expected to be about the same unless the alcohol treatment had an injurious effect on them. By referring to Table X, it will be seen that there is only a slight difference between the various lines. The average weight

of the mice not treated with alcohol was 22.4 grams and of those treated with alcohol 21.8 grams.

TABLE I

AVERAGE GAINS OF ADULT MICE

Those Not Given Alcohol

Line	Sex	No. of Mice Weighed	Average Gain in Grams at 4 Months	No. of Mice Weighed	Average Gain in Grams at 7 Months
Control	Male	3	4.6	3	4.9
Control	Female	6	4.3	6	9.3
Male alcohol	Female	6	4.3	6	6.4
Female alcohol	Male	3	5.8		
Average gain of all mice not given alcohol		18	4.6	15	7.2

TABLE II

AVERAGE GAINS OF ADULT MICE

Those Treated with Alcohol

Line	Sex	No. of Mice Weighed	Average Gain in Grams at 4 Months	No. of Mice Weighed	Average Gain in Grams at 7 Months
Double alcohol	Male	3	3.2	1	4.0
Double alcohol	Female	8	4.8	2	4.2
Male alcohol	Male	3	3.0	3	3.5
Female alcohol	Female	4	3.5		
Average gain of all mice given alcohol		18	3.9	6	3.8

Tables I and II give the average gain of the different lines for four months and for seven months. The mice that did not receive alcohol gained more than those that were treated; the former gaining 4.6 grams on an average for four months and the latter 3.9 grams; in seven months the untreated mice gained 7.2 grams and the alcoholized 3.8 grams.

It is possible that the handling of the mice and the extra exercise they took in the excitement of being alcoholized might account in part for their growing less than the untreated mice. In the 1911 experiments the control mice carried 7 months gained only 2 grams on an average while the alcohol mice gained 6 grams; in the second generation carried four months the controls gained 1 gram each and

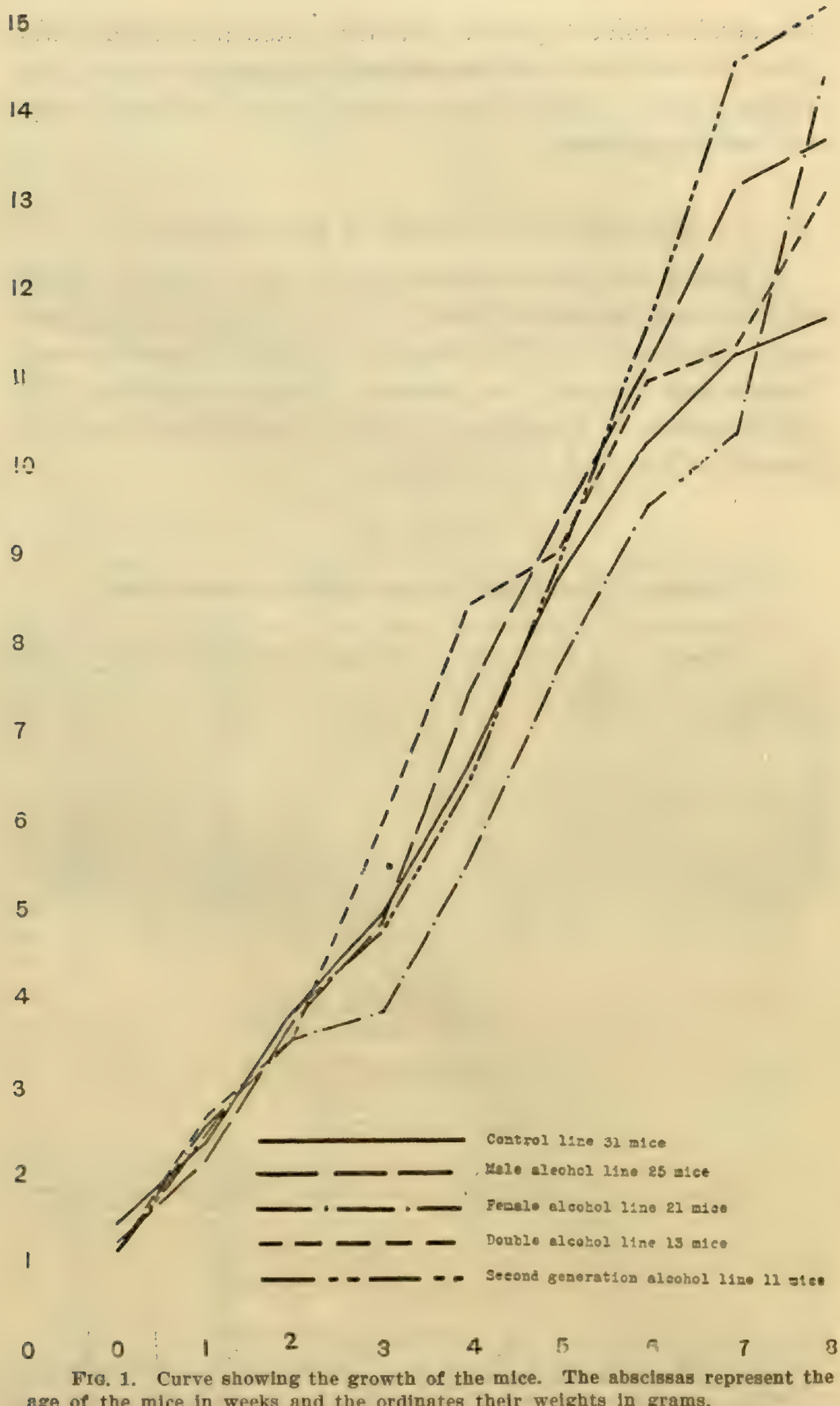

FIG. 1. Curve showing the growth of the mice. The abscissas represent the age of the mice in weeks and the ordinates their weights in grams.

the alcohol mice 2 grams. In that case the alcohol was given in the food and water and apparently had a fattening effect. None of those animals were handled except for weighing them.

RECORDS OF THE YOUNG OF EACH FEMALE

A record was kept of the young of each female. Many of the young in each of the lines were eaten by their parents. Tables III to VIII show the number of months each female was carried; the number of litters each had; the total number of young born, and the number that died apparently from lack of vitality.

TABLE III

RECORD OF THE YOUNG OF EACH FEMALE CONTROL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
A	7	1	7	0
B	7	2	10	0
C	7	1	6	0
D	7	1	5	0
E	7	1	6	0
F	7	1	2	0
*G**	4	1	6	0
*H**	3	1	5	0
Total	6:7 1:4 1:3	9	47	0

* Females *G* and *H* are second generation controls.

TABLE IV

MALE ALCOHOL LINE

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
A	7	2	12	1
B	7	1	12	0
C	7	2	10	0
D	7	1	3	3
E	7	1	5	0
F	7	3	24	0
Total	7	10	66	4

TABLE V

Female Alcohol Line

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
A	4	1	7	0
B	4	1	7	0
C	4	2	12	3
D	4	2	15	1
E	2	0	0	0
F	2	0	0	0
Total	4:4 2:2	6	41	4

Note.—Females *A*, *B*, *C*, and *D* were killed by being left in the alcohol tank too long. Females *E* and *F* were killed by accident.

TABLE VI

Double Alcohol Line

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
A	4	1	5	0
B	4	1	7	0
C	9	0	0	0
D	9	3	20	0
E	4	2	15	2
F	4	2	15	0
G	4	1	5	1
H	4	1	6	2
Total	6:4 2:9	11	73	5

* These mice from *A* to *F* were left in the alcohol tank so long one day that only *C* and *D* survived the experience. After this accident one more cage, *G* and *H*, were made up from the original stock.

TABLE VII

Second Generation Alcohol Line

Female	No. of Mo. Observed	No. of Litters	No. of Young Born	No. of Young That Died
A	2½	1	6	1
B	2½	1	10	0
C	2½	1	9	0
Total 3	2½	3	25	1

Viability of the Young

Table VIII shows the number of litters and the number of young born in each line; also the number that died from lack of vitality.

TABLE VIII

RECORD OF THE YOUNG OF EACH LINE

Summary of Tables III to VII

Line	No. of Mice	No. of Months	No. of Litters	No. of Young	No. That Died	Per Cent. That Died
Control	6 1 1	7 4 3	9	47	0	0
Male alcohol	6	7	10	66	4	6
Female alcohol	4	4	6	41	4	9.8
Double alcohol	6 2	4 9	11	73	5	6.8
Second generation alcohol	3	2½	3	25	1	4

As in my former experiments none of the control young died of lack of vitality. The alcohol lines show a small percentage of deaths—4 mice or six per cent. in the male alcohol line, 5 mice or 6.8 per cent. in the double alcohol line and 4 mice or 9.8 per cent. in the female alcohol line. The second generation alcohol lines had 1 death or 4 per cent. of all of their young. In the former experiments ('11) the fatalities were somewhat greater—9 young or 11.1 per cent. in the first generation of alcoholized mice and 7 young or 12.5 per cent. in the second generation. Stockard ('16), with his guinea pigs, had a fatality of 43 per cent. in the male alcohol line, 52 per cent. in the female alcohol line, 46 per cent. in the double alcohol line and 16 per cent. in the control line.

FECUNDITY

Table IX gives the average number of litters, average number of young, and average number in a litter for one female of the control line and one female of the male alcohol lines for seven months; for one female of the female alcohol line and double alcohol line for 4 months; and for the second generation alcohol line for 2½ months.

On account of the difference in the length of time the different lines were carried, it is impossible to make a direct comparison. However the greater fecundity of all the alcohol lines over the control line is striking. Though

TABLE IX

FECUNDITY OF THE DIFFERENT LINES

Average of One Female of Each Line

Line	Months Observed	Average in a Litter	Average No. of Litters	Average No. of Young
Control	7	5.1	1.3	6.5
Male alcohol	7	6.6	1.66	11.0
Female alcohol	4	6.83	1.5	10.25
Double alcohol	4	6.5	1.4	9.3
Second generation alcohol	2½	8.3	1.0	8.3

the control mice were carried longer than any line except the male alcohol line, they have next to the lowest number of litters—the lowest being the second generation alcohol line carried only one third as long as they. They have the fewest young of all the lines and the smallest litters. The male alcohol line can be compared directly with the control line since they were both carried seven months. They have somewhat larger litters, somewhat greater average number of litters and nearly twice as many young as the controls. It is not possible to compare them directly with the lines that were only carried four months, but since the averages of these lines are almost as high as those of the male alcohol mice, it follows that the male alcohol mice were not as fecund as the female alcohol and double alcohol lines. The female alcohol mice show the greatest fecundity of all the lines, while the double alcohol and second generation of alcohol mice come next.

The three lines in which the females were alcoholized were somewhat more fecund than the line in which the males alone were alcoholized and decidedly more so than that in which neither parent was alcoholized. These results confirm those obtained in my former work ('11) where the control mice carried 7 months had 2.2 litters or 13.3 young on an average and the alcohol mice had 2.8 litters and 16.1 young; the second generation of control mice carried 4 months had 1.5 litters and 7.1 young, while the corresponding alcohol line had 1.8 litters or 12.4 young.

Why the mice had fewer young in these experiments

than in the former is not clear. It may have been due in part to the greater fluctuations of temperature in the laboratory building used here. Whatever the reason, the control mice in these experiments after the first few months occupied themselves in growing fat instead of having young.

Stockard's results on guinea pigs are directly contrary to these; his alcoholized animals had decidedly fewer young than the control guinea pigs.

COMPARISON OF THE GROWTH OF THE YOUNG IN THE VARIOUS LINES

In comparing the weights in Table X and Curve I it should be remembered that all of these young were alcoholized except the male alcohol line and of course the controls.

TABLE X

WEEKLY GROWTH OF THE YOUNG

Line	Average Weight of Female Parents	Average Weight of Male Parents	Number of Young Weighed	Average Weight per Mouse at Birth	Average Weight at One Week	Average Weight at Two Weeks	Average Weight at Three Weeks	Average Weight at Four Weeks	Average Weight at Five Weeks	Average Weight at Six Weeks	Average Weight at Seven Weeks	Average Weight at Eight Weeks
Controls	22.6	21.5	31	1.4	2.3	3.8	4.9	6.6	8.7	10.2	11.2	11.6
Male alcohol	23.2	24.8	25	1.2	2.1	3.7	4.8	7.4	9.3	11.1	13.1	13.6
Female alcohol	20.2	21.4	21	1.1	2.5	3.5	3.8	5.5	7.7	9.5	10.3	14.3
Double alcohol	21.1	23.7	13	1.2	2.6	3.5	5.9	8.4	9.0	10.9	11.3	13
Second generation alcohol	13.5	17	11	1.1	2.4	3.8	4.7	6.4	8.8	11.5	14.5	15.1

The weights of all the lines at birth and for the first two weeks are quite similar. After that variations began. The young of the double alcohol line surpassed all for four and a half weeks, while the young of the female alcohol line fell behind all the others at the beginning of the third week and remained below up to the seventh week and at the eighth week they were next to the highest. The young of the control line, the male alcohol line and the second generation of the alcohol line grew at about the same rate up to the fifth week. At this time the weight of the male alcohol line slightly surpassed all the others; then the

second generation of alcohol mice outgrew all and continued ahead until the end of the experiment. After the sixth week there were rather wide variations and this continued as long as they were weighed. At the eighth week the weights of the different lines stood in the following order: second generation of alcohol mice 15.1 grams; female alcohol mice 14.3 grams; male alcohol mice 13.6 grams; the double alcohol mice 13 grams; and the controls 11.6 grams. In my former experiments ('11) the alcohol young surpassed the controls in the rate of growth.

Comparison of the Different Lines

The control line had the fewest young of any of the lines; they had no deaths from lack of vitality; the growth of their young was slower than that of any of the other lines except the female alcohol line.

The male alcohol line was more fecund than the controls, but less so than the other alcohol lines; their death rate from lack of vitality was four mice or 6 per cent.; the growth of their young was better than that of the controls and female alcohol lines.

The female alcohol mice were the most fecund of all the lines; their death rate was four mice or 9.8 per cent.; their growth was even slower than the control mice until the last week, when they made a large gain and outgrew all but the second generation of alcohol mice.

The double alcohol mice were slightly less fecund than the female alcohol line; five mice or 6.8 per cent. of their young died; they grew a little faster than the controls.

For the second generation of alcohol mice, two males, offspring of the male alcohol line, were mated with two females, young of the same line and one female from the female alcohol line. Thus one grandmother and all but one of the grandfathers were alcoholized, the second generation were all alcoholized after they became adult and one from birth and their young also were alcoholized. The fecundity of the second generation of alcohol mice was high; they had one death from lack of vitality, or 4

per cent. of all their young, and their young grew the fastest of all the lines.

It is a matter of regret that owing to an accident—over-alcoholization one day—the second and third generations were not carried farther. However, as far as they went, no injurious effect from alcohol is apparent in fertility, nor vigor of growth, and but a small one in viability.

From indications in our results it would seem to be dangerous to draw far-reaching conclusions from data obtained on a single species. Although this work was not carried as long as it was planned, yet as it corroborates my former experiments in practically every detail it goes to prove that mice are to a degree resistant to alcohol whether it is fed or inhaled by them. From results obtained in bacteriological laboratories it is well known that mice are very resistant little animals, in comparison to sensitive animals like guinea pigs. Mice are immune to the toxin of the tetanus bacillus. It seems reasonable to expect that an animal which is immune to such a virulent toxin might have a considerable degree of resistance to the effects of alcohol.

SUMMARY

1. The white mice given alcohol by the inhalation method gave much the same results as those that received it in their food in my former experiments.

2. The fecundity of the alcohol mice was greater than that of the control mice, as in my former study.

3. Six per cent. of the young of the male alcohol line, 6.8 per cent. of the double alcohol line, 9.8 per cent. of the female alcohol line and 4 per cent. of the second generation alcohol line died from lowered vitality, while none of the control young died. Similar results were obtained in my former experiments, except that the alcohol line had a higher death rate—11.1 per cent. in the first generation and 12.5 per cent. in the second generation.

4. The growth of the young of all the alcohol lines ex-

ceeded that of the controls, as in my former experiments. The young of the second generation alcohol line outgrew all the others.

5. There were no abortions, no still births and no monsters obtained in these experiments, nor in the former.

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LINKAGE IN LYCOPERSICUM

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THE known cases of linkage of hereditary factors in plants are not as yet so numerous but that it seems desirable to place on record all instances of this condition. With that end in view I wish to call attention to some scattered data obtained several years ago, before much was known about linkage, and presented in publications which are probably not widely circulated.

Part of the data to be considered resulted from an investigation, started by Hedrick and Booth, shortly after the beginning of the awakened interest in Mendelism, which was designed to test the inheritance of Mendelian characters in the garden tomato (*Lycopersicum esculentum* Mill.). The results were published in the *Proceedings* of the Society for Horticultural Science in 1907. Two different crosses were studied. One cross was made between two varieties which differed in one character only, viz., standard and dwarf habit of vine. The other cross was between two varieties which differed in three characters, habit of vine, shape of fruit and color of fruit. It is this second cross which gives evidence that there is a genetic linkage between the factors for habit of vine and shape of fruit.

In this latter cross the varieties used are known under the varietal names of Quarter Century and Yellow Pear. The Quarter Century variety is described as having a dwarf type of vine, red-colored fruit which is shaped like that of the common garden varieties, *i. e.*, more or less spherical. The Yellow Pear variety has a standard or spreading vine, fruit yellow in color and pear-shaped.

The first generation plants grown from this cross were standard in habit of vine, with red-colored fruit which differed in shape from either parent, being oval rather

than spherical, but not constricted like the pear-shaped fruit. The second generation gave the two parental types of fruit shape together with the heterozygous fruit shape in approximately the ratio of 1:2:1. The actual numbers obtained are as follows: 114 plants with fruit shaped like the Quarter Century parent, 208 plants with fruit shaped like that of the F_1 plants and 130 plants with pear-shaped fruit. The F_1 shape of fruit was considered to be more like that of the Quarter Century parent. Both types differed from the Yellow Pear shape by not having the constricted neck. The non-constricted type is thus incompletely dominant over the constricted.

The cross therefore received one dominant factor from one parent (standard vine) and two from the other (red and non-constricted fruit). Any linkage between the factors for vine habit and shape of fruit would in this cross be a case of spurious allelomorphism or repulsion according to the English designation of this condition.

The second generation segregated into 12 distinct categories according to the Mendelian expectancy for a trihybrid where two factors show complete dominance and one factor incomplete dominance. The actual results obtained compared with the theoretical expectancies are tabulated by Hedrick and Booth, and given here in Table I.

TABLE I

HEDRICK AND BOOTH'S DATA SHOWING THE DISTRIBUTION OF THE F_2 PLANTS FROM A CROSS IN WHICH THE PARENTS DIFFERED IN THREE CHARACTERS

Character of F_2 Plants		Found	Expected
Standard vine, Fruit Quarter Century shape,	red	48	63 9/16
	yellow	16	21 3/16
Fruit Hybrid shape,	red	148	127 1/8
	yellow	40	42 3/8
Fruit Yellow Pear shape,	red	98	63 9/16
	yellow[4]	29	21 3/16
Dwarf vine, Fruit Quarter Century shape,	red[4]	37	21 3/16
	yellow	13	7 1/16
Fruit Hybrid shape,	red	17	42 3/8
	yellow	3	14 1/8
Fruit Yellow Pear shape,	red	2	21 3/16
	yellow	1	7 1/16
	Total	452	452

[4] Parental combinations.

From the results as given it can be seen that the plants with the combinations of habit of vine and shape of fruit obtained in F_2 which duplicated the parental combinations, are more numerous than expected, whereas the two new combinations, with respect to these two factors, are less than expected. The writers observed these facts and commented upon them as follows:

> The percentage of plants which fall into each class are, however, quite different from those of Mendel. This is of importance in that it indicates the number of plants which it is necessary to grow in order to get a plant with a certain combination of characters. Theoretically, 64 plants should have included all the combinations we secured; actually, with 452 plants there is one combination with only one representative. In place of one representative, there should have been seven. Our results would indicate that it is necessary to raise seven times as many tomatoes as are theoretically necessary in order to secure a desired combination. There is apparently a method to this variation. *The tendency seems to be for the second generation hybrids to go back to the same combinations of characters as the parents, rather than to form new ones.*[1] Thus it will be seen that the tomato with Quarter Century fruit on Yellow Pear vines is less than theoretical considerations alone would indicate, while the number of tomatoes with Quarter Century fruit on Quarter Century vines is more than theory would require; the same being true for the yellow pear. Inertia seems to be a factor and the preservation of the *status quo* an object among tomatoes as among men (p. 23).

In the light of more recent investigations of factorial linkage it is recognized at once that the above statement fulfils the conditions of linkage between at least two of the allelomorphic pairs concerned. Let us then examine the data more closely to see if a clear case of linkage can be made out.

Since the deviations above and below the expectancies are about the same in both the red-fruited and yellow-fruited plants, it indicates that color of fruit is an independent factor and that habit of vine and shape of fruit are partially linked with frequent breaks in the linkage. Combining the figures for yellow and red fruit and putting the 452 plants into 6 categories instead of 12, the condensed results given in Table II are obtained.

[1] The italics are mine.

TABLE II

DISTRIBUTION OF THE F_2 PLANTS WITH RESPECT TO THEIR HABIT OF VINE AND SHAPE OF FRUIT

Categories	Found	Expected	Found	Expected	Combinations of Characters
Standard vine, spherical fruit	64	84¾	379	339	New combination
F_1 fruit	188	169½			
Pear-shaped fruit	127	84¾			Parental combination
Dwarf vine, spherical fruit	50	28¼	73	113	Parental combination
F_1 fruit	20	56½			
Pear-shaped fruit	3	28¼			New combination
Total	452	452	452	452	

These figures bring out more clearly the fact that the parental combinations are in excess while the new combinations are deficient when compared with the theoretical expectations with independent assortment. Combining the numbers of the three types of standard plants and the three of dwarfs brings out another fact, viz.: that the standards exceed the dwarfs far more than is to be expected. This result can not be accounted for on the basis of linkage, because it makes no difference whether habit of vine is or is not linked with any other factor; the ratio of the total number of standards to the total number of dwarfs should approach a ratio of 3:1, if the two characters form a simple allelomorphic pair free from any other complicating factors. The same deficiency of dwarfs was noted by these investigators in the other cross reported in both the F_2 generation and the F_3 generation from heterozygous F_2 plants. The numbers they obtained were as follows:

		Found	Expected
Stone × Dwarf Aristocrat, F_2:	Standards	2,289	2,176
	Dwarfs	612	725
Stone × Dwarf Aristocrat, F_3:	Standards	1,086	1,026
	Dwarfs	282	342

With regard to this deficiency of dwarfs Hedrick and Booth suggest that

the smaller number may be due to a lesser vigor on the part of the dwarf as compared with the standard plants, and an unconscious selec-

tion by the man pricking out the young plants from the seed boxes, of the larger, that is, the standard plants. This point had been anticipated and the workmen cautioned to take the plants just as they came, but it is against all of a gardener's training to throw aside a good vigorous plant and take one half the size.

However, in the F_3 plants given above, from F_2 segregating plants, all the seeds which were planted and lived were grown to maturity, so that the latter source of error, of unequal sampling, was avoided. Still there was the same deficiency of dwarfs.

Craig (1907), in the same publication, reports large numbers of the same cross which also showed a deviation in the second generation, of too many standard plants. He does not state whether or not an attempt was made to grow all the plants obtained from the seed planted. His figures are as follows:

		Found	Expected
Stone × Dwarf Aristocrat, F_2:	Standards	2,499	2,367
	Dwarfs	657	789
Stone × Dwarf Aristocrat, F_3:	Standards	154	155
	Dwarfs	52	51

Both Halsted (1905) and Price and Drinkard (1908) give figures on the proportions of standard and dwarf plants obtained in F_2 populations. I have tabulated their data as follows:

Halsted's Data (pp. 450–462)

	Standards	Dwarfs
Dwarf Champion × Magnus, F_2	65	20
Dwarf Stone × Golden Queen, F_2	25	5
Dwarf Stone × Extra Early Tree, F_2	14	6
Lemon Blush × Dwarf Champion, F_2	18	3
Total found	122	34
Expected	117	39

Price and Drinkard's Data (Table XI, p. 40)

	Standard	Dwarf
Dwarf Champion × Red Currant	21	3
Potato Leaf × Dwarf Champion	15	9
Total found	36	12
Expected	36	12

In these last two tabulations many of the crosses show a deficiency of dwarfs, although the results as a whole agree closely with expectations. However, the numbers are too small to place much weight upon.

In connection with another investigation I have obtained considerable data on the inheritance of this character, by simply growing the seedlings in flats from 6 to 8 weeks, and then counting the dwarfs and standards without setting the plants in the field, as in all the previous cases cited. It is not always possible to distinguish all of the two types of plants, with certainty, at this stage especially, if the plants are crowded and there are many small stunted plants. However, counting the plants at this time removes the possibility of unequal sampling when only a part of the seedlings are set in the field, and also the possibility of differential viability in the field. The distributions in 5 F_2 and 16 F_3 populations from heterozygous F_2 plants gave the following results:

	Standards	Dwarfs
Dwarf Champion × Stone, 5 F_2 populations	1,103	437
Dwarf Champion × Earliana, 1 F_2 population ...	186	67
Dwarf Champion × Stone, 12 F_3 populations ...	1,707	730
Dwarf Champion × Earliana, 4 F_3 populations ..	571	149
Total found	**3,567**	**1,383**
Expected	**3,713**	**1,237**

Here the deviation from expectation is in the opposite direction. There is an excess of dwarfs. It would seem that too many of the small plants were classified as dwarfs when they were really standards. Two of the above F_2 populations were grown longer than the others in flats which were not so crowded, so that the errors in classification, I believe, were more nearly overcome. The following results were obtained:

	Standards	Dwarfs
Dwarf Champion × Stone, F_2	268	88
Dwarf Champion × Earliana, F_2	186	67
Total found	**454**	**155**
Expected	**457**	**152**

From these data it seems justifiable to conclude that dwarfness and standardness form a simple allelomorphic pair, free from any genetically complicating factors.

I have gone to this length to demonstrate the normal behavior of this character in order to be able to correct Hedrick and Booth's data according to the proportion of dwarf and standard plants, which presumably they should have obtained if all the plants had been grown to maturity, and if there had been equal viability. Moreover, whether or not the deficiency of dwarfs which they obtained is due to unequal sampling, differential viability or some unknown cause, there is no reason to suppose that the cause, whatever it is, has anything to do with the linkage between the factors for habit of vine and shape of fruit. I have, therefore, in Table III increased the number of dwarfs to the number theoretically expected, keeping the proportion of the two different kinds of dwarfs the same with respect to shape of fruit. From Table II it can be noted that 379 standards were obtained. Theoretically the dwarfs should have been one third of this number, or 126.3. There were actually only 73. This number would have to be increased 1.73 times in order to bring the number of dwarfs up to the expected number. Combining both the standard and dwarf plants in two classes each, those with and those without constricted fruit, and multiplying these two classes of dwarf plants

TABLE III

CORRECTED DISTRIBUTION OF THE F_2 PLANTS WITH RESPECT TO THEIR HABIT OF VINE AND SHAPE OF FRUIT—CHARACTERS WHICH SHOW LINKAGE

Genetic Formulæ	Characters of F_2 Plants	1. Found	2. Expected	3. Corrected	4. Expected	Combinations of Characters
AB....	Standard vine, non-constricted fruit	252	254	252	284	New combination
Ab.....	Standard vine, constricted fruit.....	127	85	127	95	Parental combination
aB.....	Dwarf vine, non-constricted fruit...	70	85	121	95	Parental combination
ab.....	Dwarf vine, constricted fruit.....	3	28	5	31	New combination
	Total..	452	452	505	505	

by 1.73, the figures given in column 3 of Table III are obtained. The figures in this column represent the number of plants which presumably should have been obtained in the four different categories, if the expected number of dwarf plants had been obtained.

These corrected numbers can then be compared with the closest theoretical ratio where the gametes, instead of being produced in the equal proportion of 1 AB:1 Ab: 1 aB:1 ab, were produced in unequal proportions (where A and B represent the two dominant factors—standard vine and non-constricted fruit). In this case if the gametes were formed in the proportion of 1 AB:4 Ab: 4 aB:1 ab, the agreement between the corrected result and the theoretical expectation is surprisingly close.

	AB	:	Ab	:	aB	:	ab
Corrected numbers	252	:	127	:	121	:	5
Corrected ratio	50.4	:	25.4	:	24.2	:	1
Theoretical ratio (1: 4: 4: 1 gametic series)	51	:	24	:	24	:	1

It is seen that the data obtained by Hedrick and Booth give a clear indication of linkage of the factor for standard vine with that for constricted fruit and dwarf vine with non-constricted fruit. Frequent breaks in the linkage occur to form the two new combinations. On the chromosome hypothesis the data show, in this case, that crossing over occurs in 20 per cent. of the gametes formed.

TABLE IV

CORRECTED DISTRIBUTION OF THE F_2 PLANTS WITH RESPECT TO THEIR HABIT OF VINE AND COLOR OF FRUIT—CHARACTERS WHICH DO NOT SHOW LINKAGE

Genetic Formulæ	Characters of F_2 Plants	1. Found	2. Expected	3. Corrected	4. Expected	Combinations of Characters
AB....	Standard vine, red fruit.....	294	254	294	284	New combination
Ab.....	Standard vine, yellow fruit.....	85	85	85	95	Parental combination
aB.....	Dwarf vine, red fruit.....	56	85	97	95	Parental combination
ab.....	Dwarf vine, yellow fruit.....	17	28	29	31	New combination
	Total.....	452	452	505	505	

The data also show that there is no linkage between the other two combinations of factors reported, viz., vine habit and fruit color, and fruit color and fruit shape. Correcting the number of dwarfs in the same way as in Table III the results for these two combinations of factors are given in Tables IV and V.

TABLE V

CORRECTED[2] DISTRIBUTION OF THE F_2 PLANTS WITH RESPECT TO THEIR SHAPE OF FRUIT AND COLOR OF FRUIT—CHARACTERS WHICH DO NOT SHOW LINKAGE

Genetic Formulæ	Characters of F_2 Plants	1. Found	2. Expected	3. Corrected	4. Expected	Combinations of Characters
AB....	Non-constricted, red fruit	250	254	289	284	Parental combination
Ab.....	Non-constricted, yellow fruit.....	72	85	84	95	New combination
aB.....	Constricted, red fruit	100	85	101	95	New combination
ab.....	Constricted, yellow fruit.....	30	28	31	31	Parental combination
	Total.....	452	452	505	505	

From these two tabulations it will be seen that the agreement between expectation and observation, when the number of dwarfs is increased to the number expected, is reasonably close, and the deviation from the expected is not such as to suggest linkage between any of these factors.

Both Halsted, and Price and Drinkard, in the publications previously mentioned, give a large number of crosses of tomatoes where the inheritance of many different characters are studied. Unfortunately, in most cases the data are presented in such a way as to show the inheritance of only one character pair at a time.

Halsted gives a dihybrid cross between two varieties differing in habit of vine—standard (A) and dwarf (a), and margin of leaf—serrate (B) and entire (b). The

[2] Since habit of vine is not concerned in this cross it is, of course, unnecessary to correct for the low number of dwarfs as in the two previous tables. I have done so simply to show that it does not affect the goodness of fit to any great extent.

cross was made in such a way that one dominant factor entered from each parent. The numbers obtained

	AB : Ab : aB : ab
Found	49 : 16 : 13 : 7
Expected	48 : 16 : 16 : 5

do not indicate any linkage between these factors.

Price and Drinkard's data indicate that there is no linkage between shape of fruit and color of fruit in two different crosses (agreeing with the data given in Table V), none between foliage color and fruit color, and none between foliage color and fruit shape. In these crosses the numbers are too small to be sure of the conclusions with regard to linkage. They give the results of a cross, however, which shows complete linkage between green foliage color and two-celled fruit, as opposed to yellow foliage color and many-celled fruit. Only 24 F_2 plants were grown, which were of two types only, duplicating the parents.

These characters, foliage color and loculation of ovary, can not be the expression of the same factor because many varieties are known with these characters combined in the other ways. In fact the majority of the common garden varieties have green foliage and many-celled fruit. Neither does it seem probable that these dissimilar characters form a series of multiple allelomorphs as some cases of complete linkage, for instance, cob and pericarp colors in maize, are considered to be. Although the number of plants is small, as the writers state, it would seem that among 24 plants at least one new combination would appear if the factors were independent of each other. Larger numbers of a similar cross, studied by back crosses in the more favorable way, will probably show these factors to be partially linked.

Crane (1915) reports a cross between varieties of tomatoes differing in rather complex characters of inflorescence and fruit shape. He obtained figures which indicate partial linkage in these characters, but states that "the

numbers are not sufficiently large to form any conclusion as to the intensity of the coupling, nor to establish the existence of the same with certainty.''

A number of clearly segregating characters are known in the tomato. Halsted lists 7 alternative unit character pairs, while Price and Drinkard give 13. However, from their own statements in regard to the behavior of these characters, and from my own rather limited experience with tomatoes, the number of different character pairs which they list should be reduced. For instance, only two allelomorphic pairs are known for color of fruit, viz., red and yellow flesh or endocarp, and yellow and colorless fruit skin or epicarp, while Price and Drinkard give four, and Halsted three, character pairs of fruit color. Different combinations of skin colors and flesh colors give the different colored fruits. For example, colorless epicarp over red endocarp gives pink-colored fruit.

TABLE VI

MENDELIAN CHARACTERS IN THE GARDEN TOMATO

(Revised from the lists given by Halsted and by Price and Drinkard.)

		Dominant	Recessive
Fruit shape	1	Spherical (non-constricted)	Pyriform (constricted)
Fruit shape	2	Roundish conic	Roundish compressed
Loculation of ovary	3	Bilocular	Plurilocular
Endocarp color	4	Red	Yellow
Epicarp color	5	Yellow	Colorless
Fruit surface	6	Smooth	Pubescent
Vine habit and leaf surface	7	Standard; Smooth	Dwarf; Rugose
Leaf margin	8	Serrate (normal or fine leaf)	Entire ("potato" or coarse leaf)
Leaf type	9	*Pimpinellifolium* type	*Esculentum* type
Foliage color	10	Green	Yellow
Inflorescence type [3]	11	Simple	Compound

It is somewhat uncertain as to the number of independent factors concerned in fruit shape. According to Crane (*loc. cit.*) and Groth (1912 and 1915) there are a number of factors and it is not always possible to distinguish between the various shapes. There is apparently a corre-

[3] See Crane, 1915, p. 4.

lation between the loculation of the ovary and some fruit shapes, although not necessarily with the constricted type of fruit. The foliage characters (Groth, 1911) are rather complicated. Also the color of foliage and the color of the epicarp of the fruit may be associated in the same way that habit of vine and leaf surface are, *i. e.*, the expressions of one factor. Dwarf plants always have a more rugose foliage than standard plants. According to Groth (1915, p. 17) dwarfness can not be associated with pubescent fruit for some reason.

A list of the Mendelian genes, so far known in the tomato, is given in Table VI.

The list is only tentative. A more detailed study of these characters will probably necessitate further revision. Other character differences may be known and should be added. There are, however, at least 10 plainly segregating genes and probably more. The behavior of 6 of these with respect to their being linked or not linked with each other, in all the 15 possible combinations,[4] is known in the case of 7 of them and can be predicted for 5 others. These 15 combinations with respect to linkage are summarized as follows:

CHARACTERS SHOWN TO BE LINKED FROM THE DATA OF HEDRICK AND BOOTH, AND PRICE AND DRINKARD

Vine Habit,	**7**	**with**	**Fruit Shape,**	**1**
Foliage Color,	**10**	**with**	**Loculation of Ovary,**	**3**

CHARACTERS SHOWN NOT TO BE LINKED FROM THE DATA OF HEDRICK AND BOOTH, HALSTED, AND PRICE AND DRINKARD

Vine Habit,	**7**	**with**	**Endocarp Color,**	**4**
Vine Habit,	**7**	**with**	**Leaf Margin,**	**8**
Fruit Shape,	**1**	**with**	**Endocarp Color,**	**4**
Fruit Shape,	**1**	**with**	**Foliage Color,**	**10**
Endocarp Color,	4	with	Foliage Color,	10

[4] The possible number of combinations is obtained from the formula $\frac{n^2 - n}{2}$ where n^2 equals the total number of combinations, two at a time, between n different units but no factor can, of course, be paired with itself and the remaining pairs are duplicated.

CHARACTERS WHICH CAN NOT BE LINKED (ON THE CHROMOSOME HYPOTHESIS IF THE ABOVE CASES HOLD TRUE)

Endocarp Color,	4	with	Loculation of Ovary,	3
Vine Habit,	7	with	Loculation of Ovary,	3
Vine Habit,	7	with	Foliage Color,	10
Fruit Shape,	1	with	Leaf Margin,	8
Fruit Shape,	1	with	Loculation of Ovary,	3

CHARACTERS WHICH MAY OR MAY NOT BE LINKED

Leaf Margin,	8	with	Loculation of Ovary,	3
Leaf Margin,	8	with	Endocarp Color,	4
Leaf Margin,	8	with	Foliage Color,	10

Since not all the possible combinations of the 6 factors have been tested, and 4 of the factors have not been tested at all, either in combinations among themselves or with any of the other 6 factors, the possibilities of linkage in the tomato have only begun to be examined. It is noteworthy that none of the 7 combinations which either do or do not show linkage are at variance with the interpretation of linkage according to the chromosome hypothesis. For instance, where one of two linked genes is unlinked with a third, the other linked gene is also unlinked with it. This is a necessity on the chromosome hypothesis.[5]

To fit the facts to the chromosome hypothesis it is only necessary to assume that genes 1 and 7 are located in one chromosome which we may call A; genes 3 and 10 must be located in another chromosome, B; gene 4 must be located in a third chromosome, C. Gene 8 can not be in A but may be located in B, C or a fourth chromosome. With these assumptions all the data so far obtained fall into line and if these data are substantiated the other results predicted must hold if the chromosome hypothesis is correct. It must be noted that many of the cases cited here are not fully established on account of the small numbers, and furthermore there is the possibility that what is taken to be independent assortment may be crossing over of about 50 per cent.

[5] This may also be a necessity on the reduplication hypothesis or may even be axiomatic and must hold for any and every hypothesis that might be put forth to account for factorial linkage.

Since the chromosome number is comparatively low ($1n = 12$, Winkler, quoted after East, 1915) the tomato is rather favorable plant material in which to study linkage.

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GENETICS VERSUS PALEONTOLOGY

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ALTHOUGH the title of this article has a somewhat controversial sound, its purpose is merely to discuss, in a perfectly frank and appreciative way, certain passages in the recent works of two eminent geneticists, Professor William Bateson and Professor T. H. Morgan.

"Naturally," says Professor Bateson,[1] in describing a certain theoretical *impasse* as regards the method of evolution, "we turn aside from generalities. It is no time to discuss the origin of the Mollusca or of Dicotyledons while we are not even sure how it came to pass that *Primula obconica* has in twenty-five years produced its abundant new forms almost under our eyes."

Taken in connection with other passages, this seems to imply the belief that the present is no time to investigate phylogenetic problems or to formulate any generalities concerning the origin of systematic groups of organisms. Until the facts of heredity are explained we should turn aside from most of the major problems that engaged the attention of the great comparative anatomists and paleontologists of the nineteenth century. The origin of paired limbs, the origin of the vertebrates, the mutual relations of the great phyla of invertebrates, and similar phylogenetic problems in botany, all these and hundreds more of the same category having been laid aside by the majority of zoologists, are dead or moribund subjects which a student of genetics had better leave in decent obscurity. If Professor Bateson had said "I turn aside from generalities. I have no time to discuss the origin of the Mollusca or of Dicotyledons. I used to be interested in such things, but now I would much rather study the mutations of *Primula obconica*," nobody could reasonably object; but when he says "*we* turn aside from generalities. It is no time [for any one] to discuss the origin of the

[1] *Science*, N. S., Vol. 40, 1914, p. 294.

Mollusca . . .,'' etc., he is apparently mistaking a part for the whole, and also confusing two fairly distinct lines of investigation, genetics and phylogeny.

As long as museums and universities send out expeditions to bring to light new forms of living and extinct animals and new data illustrating the interrelations of organisms and their environments, as long as anatomists desire a broad comparative basis for human anatomy, as long as even a few students feel a strong curiosity to learn about the course of evolution and the relationships of animals, the old problems of taxonomy, phylogeny and evolution will gradually reassert themselves even in competition with brilliant and highly fruitful laboratory studies in cytology, genetics and physiological chemistry. Very likely the fortunate few who gain some first-hand knowledge in all these fields will realize that such problems as the origin of the Mollusca or the origin of the Dicotyledons have as much vitality as the problem of the origin of the earth or the problem of the phyletic relationship of man with the lower animals.

The student of the evolution of the vertebrates may well reserve judgment as to theories of evolution, and he must even confess his inability to trace a detailed phylogenetic succession except for short intervals; yet he is well assured, from long experience with the paleontological record and with the comparative anatomy of recent animals, that he can trace in a general way the history of many groups and of many structures, and he should know very definitely where the evidence is fairly complete and where it is weak and lacking. In view of the wealth and detailed character of the evidence (which is hardly known except to a limited number of specialists) no competent authority would doubt, for example, that all the races of modern Equidæ, walking on the tips of their one-toed feet, have been derived from three-toed *Hipparion*-like forms, or that these in turn lead back to *Eohippus*-like forms of the Eocene, with four digits on each forefoot and three on each hind foot; or that during the Tertiary Period the molar teeth of horses (in the broadest sense) changed

from low-crowned teeth of simple pattern into long-crowned teeth of a complex pattern. And even in the practical absence of paleontological evidence it is sufficiently established that the Cetacea, which are now of pelagic habit and fish-like habitus, represent transformed terrestrial or littoral quadrupeds, which at a remote epoch were placental mammals of some sort. Nor can it be justly doubted that birds are "glorified reptiles," that bats are volant derivatives of arboreal mammals, that teleosts have been derived from ganoids. Detailed knowledge of the evidence in hundreds of such cases leads the paleontologist to say with considerable confidence: "this later type of animal has probably been derived from that earlier type; this structure has undergone such and such changes during certain geological periods."

Professor Bateson is equally cold towards outworn notions about adaptation. "Naturalists may still be found," he says,[2] "expounding teleological systems which would have delighted Dr. Pangloss himself, but at the present time few are misled. The student of genetics knows that the time for the development of theory is not yet. He would rather stick to the seed-pan and the incubator."

Two very distinct ideas seem to be implied in this passage and the context, first the rejection of the supposed principle of progressive adaptation in evolution, and secondly the idea that conclusions regarding evolution should be limited to those in which control experiments can be made.

As to the principle of progressive adaptation, it is an indisputable fact that existing animals possess structures which are highly efficient in the performance of certain functions, *e. g.*, the locomotive apparatus of the horse, effective for progression over hard ground; its masticatory apparatus, effective in the trituration of siliceous vegetation. Paleontologists, after studying the phylogenetic history of such structures, must infer that progressive advance of structure has been influenced to a

[2] *Ibid.*, p. 293.

greater or lesser degree by environmental conditions. It is certain that changes in the conditions of life are not the sole causes of modification, it is highly probable that the chromosomes are insensitive to most somatic reactions to the environment; yet how can the student of the Cetacea, who sees how thoroughly the ancestral quadrupedal heritage has been overlaid by the fish-like habitus, doubt that in the end, and perhaps in some very indirect way, the pelagic environment has conditioned the line of evolution of the cetacean chromosomes, as it plainly has conditioned the evolution of cetacean cytoplasm. And when similar adaptations are produced among widely separate stocks, it can scarcely be doubted that the similar results are due to the similarity of the external conditions as well as to the fundamental similarities of all cytoplasm and of all chromatin. Hence, without any commitment as to the mode of evolution, paleontologists adopt the principle of progressive and retrogressive adaptation to environmental conditions as sufficiently demonstrated. And most paleontologists would probably recognize that the foot, for example, is just as much a part of the environment of the femur as is the medium upon which the foot rests, in other words that evolution of a given structure is conditioned by its internal environment as much as by external environment.

Yet such is the skepticism which sometimes results from modern studies in genetics that I have known graduate students who seriously doubted the reality and value of the principle of progressive and retrogressive adaptation, on the ground that, as natural selection and the inheritance of "acquired" characters had both been disproved, there was no conceivable means whereby adaptation could be brought about! But if these skeptics would study for example the evolution of Triassic ganoids into Cretaceous and modern teleosts, if they would consider in detail the structural improvements in the locomotive apparatus of teleosts, which involve the transformation of scales into dermal rays, or of a heterocercal tail into a homocercal tail, or if they would examine the evidence

bearing upon the evolution of the paired limbs or upon the evolution of the vertebrate skull, or of the carnassial teeth of Carnivora, they would, I believe, be forced to accept the principle of the progressive efficiency of structures for special functions as at least a fruitful working hypothesis.

A distrust of the word "adaptation," which has been in the bad company of the Lamarckian theory, is apparently revealed in Professor T. H. Morgan's "A Critique of the Theory of Evolution" (1916). The author, however, apparently favors the idea of natural selection operating upon "advantageous" or "beneficial mutations" and eliminating the "injurious effects" of other mutations. Of course if "adaptation" really implied an acceptance of the Lamarckian theory it would be better to use some such phrase as "progressive functional adjustment," but the important point to bear in mind is that nature has produced myriads of structures which have a very definite functional adjustment with other structures, in other parts of the body, or with parts of other bodies, or with parts of the environment. And it is perfectly plain from the evidence of comparative anatomy and paleontology that functionally correlated parts have often evolved together, and with definite reference to each other, let the explanation of that fact be what it may. Professor Morgan himself has fully recognized this fact in his address[3] entitled "Chance or Purpose in the Origin and Evolution of Adaptation."

The second idea which seems to be implied by Professor Bateson, and which I have heard certain university students express, is that phylogenetic "speculations" are unverifiable, because "control experiments" are not possible. By similar reasoning geological theories concerning the history of the earth, archeological theories concerning the history of peoples, and all historical studies based upon internal or circumstantial evidence are equally untrustworthy. The answer to such a theoretical objection, if it were definitely made, would be that comparative

[3] *Science*, Vol. 31, 1910, pp. 201–210.

anatomy, geology, phylogeny, etc., are practical arts which have to be learned by experience. Phylogenists must constantly distinguish between primitive and specialized characters, and if their experience, caution and judgment be adequate they may be as successful as physicians are in diagnosis. Of course physicians make mistakes and so do phylogenists, but in the long run both succeed in sifting the false from the true, even without the aid of direct experimentation. Nature herself often provides control experiments, as when she forces animals of widely different stocks into similar life habits, or when she takes a primitive type of skull and dentition and molds them into a wide variety of adaptive types, meanwhile preserving the original pattern as a "control," either in the form of a "living fossil," persisting in a primitive environment, or in the form of a real fossil found in Tertiary strata.

Professor Morgan makes a serious and important criticism of the comparative anatomical and paleontological doctrine that structures have been derived by progressive continuous stages. He is evidently inclined to think that structures have rather been derived through discontinuous mutational stages. It would be easy, he shows, to arrange a graded series of fruit flies belonging to distinct mutations, having at the one extreme perfectly formed wings and at the other extreme no wings at all. But this series by no means represents the historical order of appearance of these mutants, which are not genetically derived one from the other, but have arisen independently. Again (p. 13)

> . . . it is easily possible beginning with the darkest eye color, sepia, which is deep brown, to pick out a perfectly graded series [of races] ending with pure white eyes. But such a serial arrangement would give a totally false idea of the way the different types have arisen; and any conclusion based on the existence of such a series might very well be entirely erroneous, for the fact that such a series exists bears no relation to the order in which its members have appeared.

"Suppose," he continues, "that evolution 'in the open' had taken place in the same way, by means of *discontinuous* variation. What value then would the evidence [for

evolution] from comparative anatomy have in so far as it is based on a continuous series of variants of any organ?''

We may readily admit that *if* evolution in the open has taken place through discontinuous variation, the supposed evidence for evolution based on continuous series of variants is valueless. But neither Professor Morgan nor the present writer try to persuade students of the truth of evolution upon the ground that supposedly continuous series have been traced purporting to illustrate the evolution of *single* structures. As he well intimates, the strongest evidence for evolution is the fact that all the widely diverse members of each group exhibit a common heritage or ground-plan of homologous structures. When that common ground-plan is recognized and when the probable habits of the ancestral form are clearly perceived a long step has been taken toward deciphering the evolutionary history of the group; and it will often be easy to decide what characters and habits have been lost and what new ones have been acquired.

Whether we think evolution has taken place by means of discontinuous variation or through regular progressive and continuous series one of the chief aims of zoologists is, or should be, to discover the facts concerning the phyletic interrelationships of groups and the evolution of their habits and structure. And often the chief earlier and later stages might be recognized in spite of ''discontinuous variation.'' For example, if one knew nothing about the history of the mutant races of *Drosophila* it would seem a safe inference that the apterous form had been derived eventually from a winged type, because a comprehensive study of Diptera in general would indicate that wingless flies were degenerate and not primitive in that respect. Similarly if the systematic relationships and probable derivation of *Drosophila* were given due consideration the races with imperfect eyes and those with duplicated parts would naturally be regarded as degraded or aberrant, rather than original or primary types; and if many intermediate stages between winged and wingless

forms were found living at the same time in a restricted area one might perhaps have suspected that these contemporaneous intermediate forms were parallel offshoots of a normal parent stock rather than linear descendants one of another.

It may well be true that, until it can be shown that evolution "in the open" is continuous and not discontinuous, all "laws" and "principles" which merely assume such continuity are open to question. But there is considerable evidence for the conclusion that many races of mammals have evolved either quite continuously or by small *successive* gradations. It is true that in some cases apparently new and distinct forms also appear in successive horizons, but these new forms may be immigrants from other distribution centers[4]—the little-modified descendants of indigenous races being often found side by side with their more progressive immigrant relatives.

The great collections of American Eocene and later mammals which have been brought together by the systematic explorations of the American Museum of Natural History are all exactly recorded as to level, so that except in a few instances there can be no doubt whatever as to the chronological sequence of the specimens. These collections, numbering many thousands of specimens, are being minutely studied by several investigators, who are not trying to prove any theory of evolution, but are recording and identifying specimens and analyzing their observations, with such accuracy and judgment as they may have gained from twenty years of experience in this work.

The results of these studies, as bearing on the question of continuity *vs.* discontinuity in evolution, are too extensive and complex to be summarized here, but a few examples may serve to illustrate the kind of evidence available and the conclusions which have been drawn in typical instances.

Very often as we pass from lower to higher strata of

[4] Matthew, W. D., "The Continuity of Development," *The Popular Science Monthly*, Nov., 1910, pp. 473–478.

a given formation the successive species show a regular increase in size and a progressive molarization of the pattern of one or more of the premolar teeth. A typical case of this kind is recorded by Matthew[5] in the genus *Cynodontomys*, a small insectivorous mammal of the Lower Eocene which is represented by three successive species which do not overlap in time, but are separated by small progressive differences in the premolars and molars. Each species is represented by series of from ten to twenty specimens, from successive horizons of the Bighorn and Wind River Basins in Wyoming. Another instance of practically continuous evolution is furnished by the Middle Eocene titanothere *Palæosyops*. Professor Osborn and the present writer have observed that in this genus the species named *paludosus*, *major*, *leidyi* and *robustus* form a regular and nearly continuous series extending from the lower to the higher levels of the Bridger Basin, in which the lower and upper premolars gradually evolve toward the molar pattern. A fifth species, *P. copei*, from the uppermost fossiliferous levels of the Bridger Basin is considerably more advanced than any of its predecessors, and is connected with them by intermediate specimens from the nearby Washakie Basin of the same age.

In other cases the material indicates that while some phyla evolve at a nearly uniform rate others lag behind at varying rates, the extreme cases furnishing the relicts or "living fossils" which give so many useful hints as to the primitive characters of a race.

Such an instance is furnished by the history of the Eocene primates *Pelycodus* and *Notharctus* (Table I). The oldest species, *Pelycodus ralstoni*, is of small size and very primitive character. The latest species, *Notharctus crassus*, is about twice as large and of very advanced character. Many intermediate stages are known. Of these *P. relictus* is an extremely conservative form which has acquired only a few of the progressive characters seen in its contemporaries.

[5] *Bull. Amer. Mus. Nat. Hist.*, Vol. XXXIV, 19, p. 470.

TABLE I

PROGRESSIVE INCREASE IN THE LENGTH OF THE LOWER MOLARS (m_{1-3}) IN LOWER AND MIDDLE EOCENE LEMUROIDS OF THE FAMILY ADAPIDÆ (SUBFAMILY NOTHARCTINÆ)

Data for Lower Eocene species compiled from Matthew (*Bull. Amer. Mus. Nat. Hist.*, Vol. XXXIV, 1915, p. 436. Data for Middle Eocene species by Granger and Gregory.

MIDDLE EOCENE	HORIZONS UPPER BRIDGER Bridger Basin, Wyo.	pm^4 with two large external cusps, m^{1-3} with large mesostyle, molars quadritubercular.							*N. crassus* 20.7–23.5 mm.
	LOWER BRIDGER Bridger Basin, Wyo.	*P. relictus* 15	*N. formosus* 16.6	*anceps* 17.5	*affinis* 18	*tyrannus* 18 est.	*tenebrosus* 18.6	*rostratus* 19	*pugnax* 20.7
LOWER EOCENE	LOST CABIN Wind River Basin, Wyo.				*Notharctus nunienus* 15.5				*N. venticolus* 18–19.2
	ALMAGRE* (San Juan Basin, N. M.) LYSITE Bighorn Basin, Wyo.				*Pelycodus frugivorus* 14–16				*P. jarovii* *P. tutus* 19
	UPPER GRAY BULL Bighorn Basin, Wyo. LOWER				*P. frugivorus* *P. trigonodus* 15 *P. ralstoni*				*P. jarovii* 16–18 *P. jarovii* (rare)
	SAND COULÉE Clark's Fork Basin, Wyo.				*Pelycodus ralstoni* 11–14 mm.				pm^4 with 1 external cusp, m^{1-3} without mesostyle, molars tritubercular.

* The upper levels of the Almagre of New Mexico are perhaps equivalent to the Lysite. Granger, *Bull. Amer. Mus. Nat. Hist.*, Vol. XXXIII, 1914, p. 207.

In certain cases the paleontological evidence is indecisive, as between the hypothesis of successive mutation *in loco* and the hypothesis of continuous evolution in an unknown center of evolution followed by discontinuous immigration of later stages into the region under observation. Such a case is described by Matthew[6] as follows:

[6] *Bull. Amer. Mus. Nat. Hist.*, Vol. XXXIV, 1915, p. 316.

Osborn in 1902 pointed out the evolutionary progress observable in the species of *Hyopsodus* from successive stages of the Lower and Middle Eocene; this is in general confirmed and extended by the far larger collections [comprising more than a thousand specimens] now available and the somewhat wider geologic range of the genus; but it is evident that not one but three or four phyla are present in each horizon; the relations of the Lower Eocene species to those of the Middle Eocene are not wholly clear, and the geological overlap of stages of each structural phylum suggests rather progressive displacement of older by newer stages coming in from some other region, than gradual evolution *in loco*. It might equally well be interpreted as the displacement of older by newer "mutants," in the DeVriesian sense of the term.

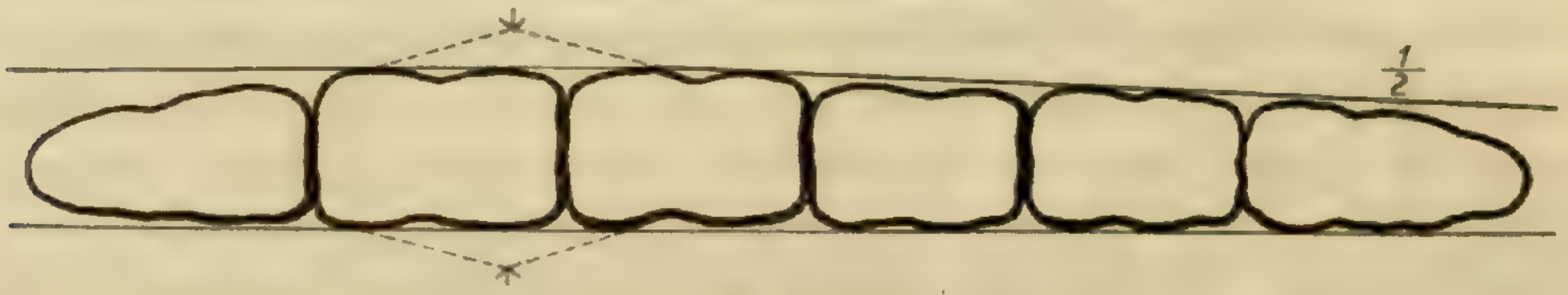
However this may be, the Lower Eocene species are distinguished from those of the Middle Eocene by the less molariform premolars, and this is most noticeable in *H. simplex* from the lowest horizon, while the Lost Cabin species [from the upper part of the Lower Eocene] approach nearest to those of the Bridger [Middle Eocene].

Examples of this kind might be multiplied, tending to show that the evolution of Tertiary mammals has often been more or less continuous, or by small successive changes, at least during the relatively brief geological periods that are represented by a large series of specimens from closely sequent levels of an uninterrupted stratigraphic series. And although mutations may well be a paleontological reality, there is little danger that vertebrate paleontologists are likely to draw false inferences regarding the history of structures and of races through mistaking independent contemporaneous mutants for successive stages, for the simple reason that their observations are based on long series of specimens which are arranged in their true chronological sequence, from ascending geological horizons covering the whole Tertiary Period.

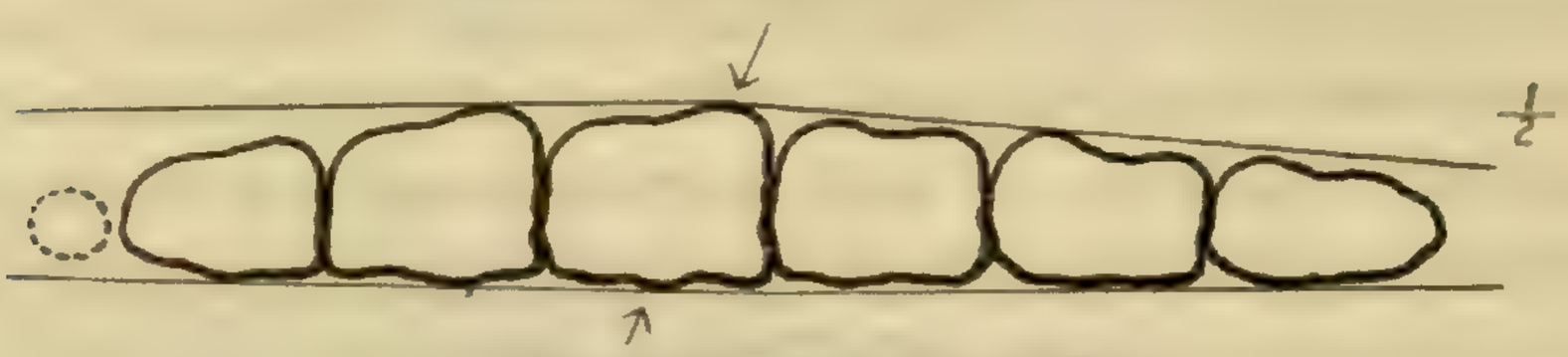
In this connection I submit an accurate diagram (Fig. 1) by Mr. Granger, which is fairly representative of the kind of evolution demonstrated among many, but not all, known races of mammals during the Tertiary and Quaternary Periods, a period of time conservatively estimated at 4,000,000 years.

The character of the evidence tending to show that the paleontologist is dealing with truly successive stages and

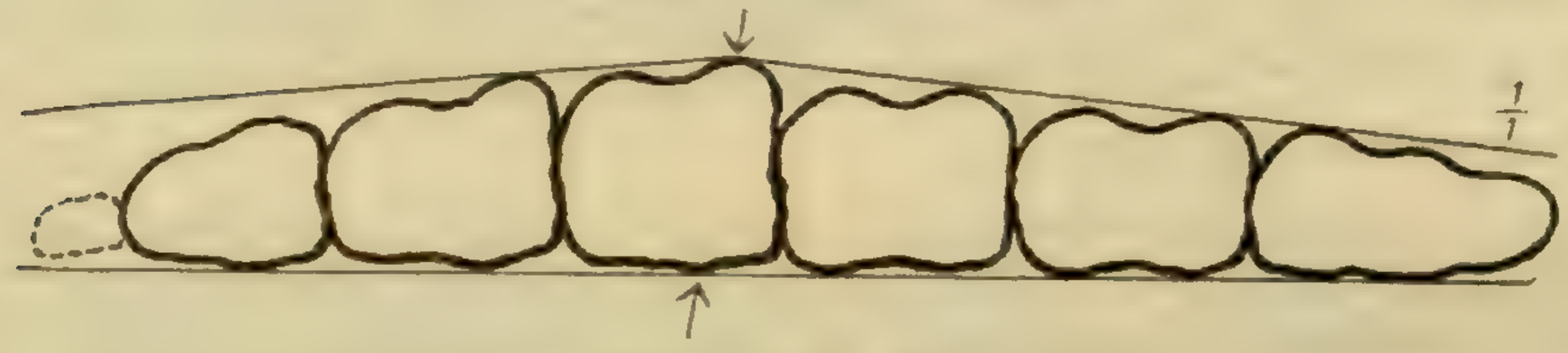
Equus caballus Recent.



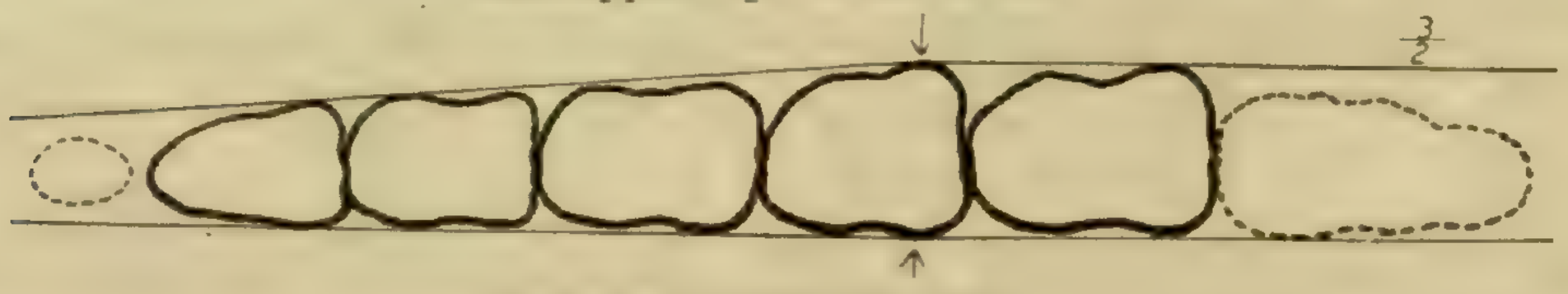
Equus complicatus Pleistocene.



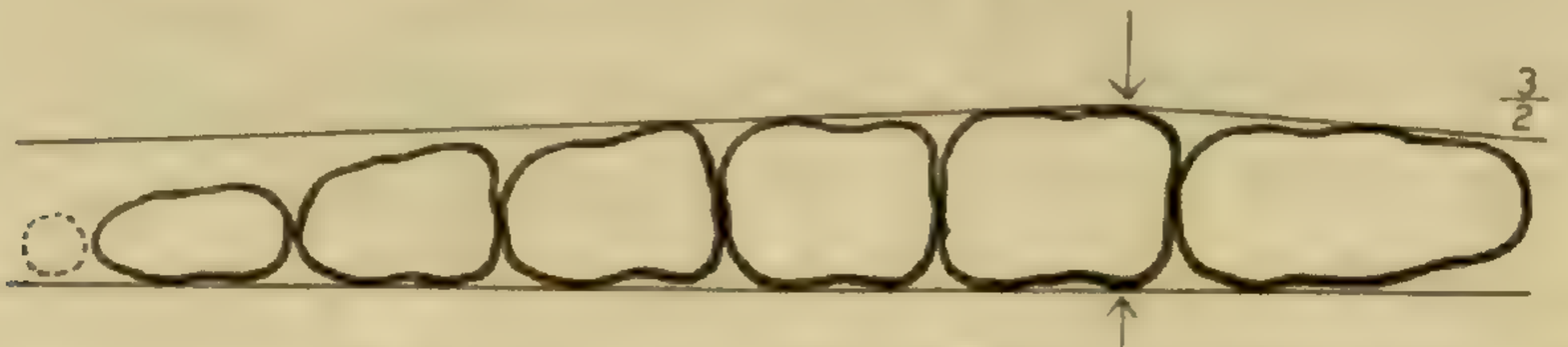
Hypohippus sp. Loup Fork.



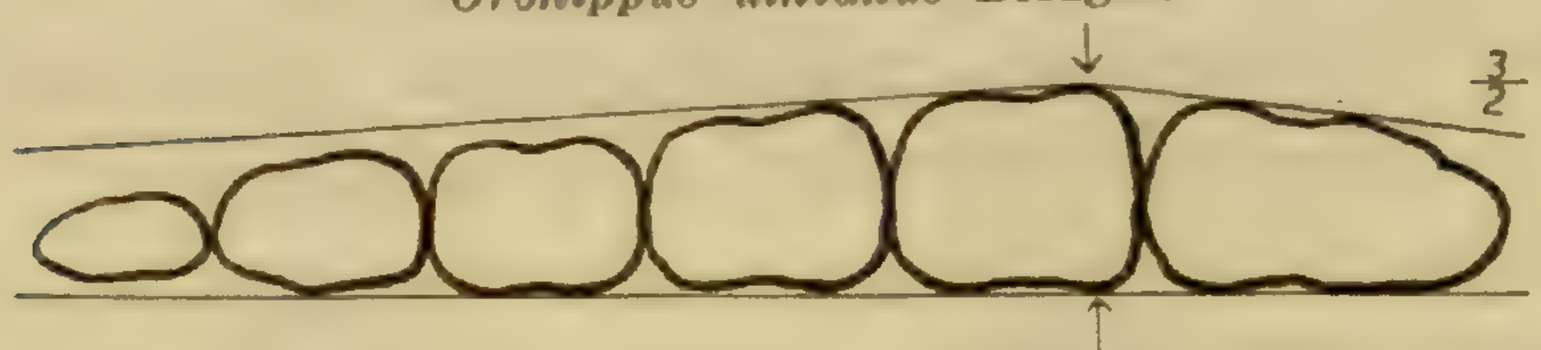
Mesohippus sp. White River.



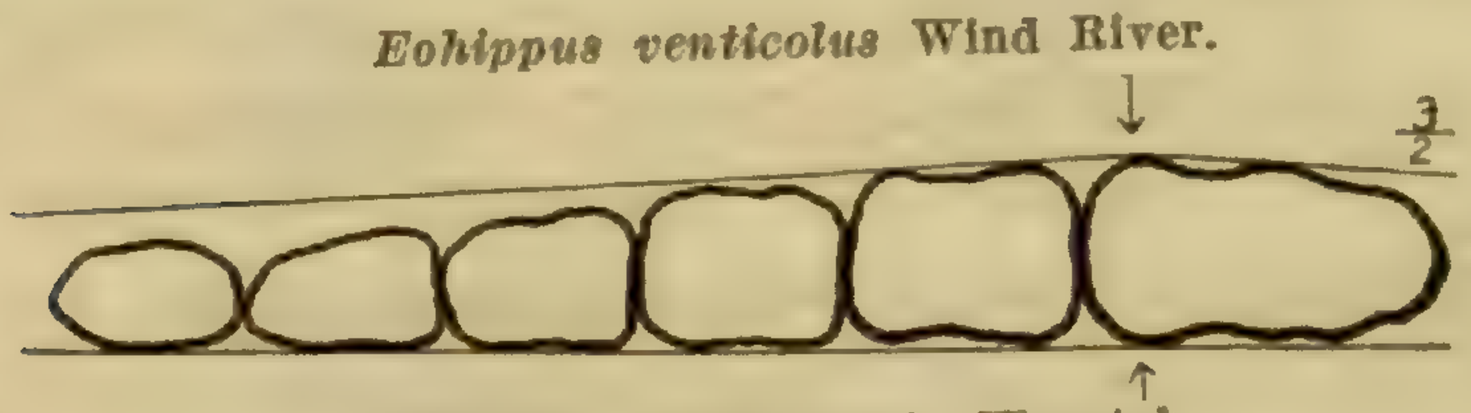
Epihippus uintensis Uinta.



Orohippus uintanus Bridger.



Eohippus venticolus Wind River.



Eohippus cristonensis Wasatch.

FIG. 1. Accurate outlines of lower cheek teeth of Equidæ. Arrows indicate point of greatest transverse diameter in each series, ranging from the third molar in the Wasatch species to the third premolar in the modern Equidæ. After W. Granger (*Bull. Amer. Mus. Nat. Hist.*, Vol. XXIV, Art. XV, pp. 221–264, 1908.)

not with an arbitrarily selected series of mutants is further illustrated in the following note by Dr. Matthew.

Of the hundreds of specimens examined no horse from the Lower Eocene has ever been found which had *any* fully molariform premolars. No horse out of the Middle Eocene has either more or less than *one* molariform premolar in the lower jaw, on each side. Out of the Upper Eocene all horses have *two* molariform premolars. In the Oligocene all have *three.* All Oligocene and older horses have brachyodont molars without cement. All Miocene horses are progressively hypsodont with a progressive increase in the amount of cement. The milk teeth of Miocene horses have almost no cement. Those of all Pliocene and later horses are heavily cemented. At each successive stage of evolution the cement appears at an earlier stage in the ontogeny of the tooth. These are simply a few out of many progressive changes in the teeth, and they are accompanied by equally clear progressive changes in the skull and skeleton. Every one of these progressive stages is as exactly limited in time as the ones cited.

Geneticists who are examining the nature of the paleontological evidence regarding modes of evolution would do well to realize that only a small part of the available material bearing on the subject is either exhibited or published. The scientific staff of the American Museum of Natural History would be very glad to exhibit to their colleagues the great wealth of accurate data, concerning the chronological sequence of specimens, which has been gathered during twenty years of close exploration; they would also be pleased to place before them any of the extensive series of specimens, sometimes amounting to several thousands of individuals, which appear to throw light upon the problem of continuity *vs.* discontinuity.

In conclusion, paleontologists can show that evolutionary changes have involved *progressive and measurable emphases or suppressions of earlier structures or of earlier proportions* (allometric evolution, Osborn); and when the progressive emphases are manifested as focal outgrowths they seem like "new" structures (rectigradations, Osborn). Paleontologists, however, are not in a position to say which characters would be transmitted according to the Mendelian ratio, nor can they prove what were the cytological causes of the evolutionary changes

which they record or infer. In that direction lies opportunity for consultation with the men who study enzymes, chromosomes, heredity and variation.

The Batesonian hypothesis that both the progressive differential emphases or suppressions of organs, and the focal outgrowth of new structures, have been due to a secular, differential stopping down of inhibitory factors inherent in the germ-cells seems to the present writer quite consistent with the observed facts of evolutionary change; but apparently no observations that the paleontologist can make could furnish any critical tests of this hypothesis; it therefore has for him a stimulative philosophical value, but hardly constitutes a working hypothesis for the discovery of new facts and principles in his limited field.

The nature of later events being determined in part by the nature of their precedent events, no matter how many causal series may be interwoven in the final outcome, it follows that paleontologists, like other historians, contribute to a partial understanding of existing conditions merely by arranging past events in their true chronological sequence. The characteristics of existing Cetacea are determined in part by the germinal and somatic characteristics of their remote quadrupedal ancestors, as well as by the conditions of the pelagic life into which they somehow drifted; so too the characteristics of man, as a bipedal, bimanous, anthropoid Primate are determined in part, as I believe, by the fact that the remote ancestors of the man-greatape stock were arboreal, quadrumanous, lemuroid Primates of the Lower Eocene.

For such reasons, I must continue to hold that "progressive adaptation" when cleared of all implications as to the mode of evolution, stands for a historical and verifiable process; that the time for developing phylogenetic conclusions and for revising comparative anatomy and classification is always *now*, as fast as the evidence can be gathered and analyzed.

SHORTER ARTICLES AND DISCUSSION

STUDIES ON INBREEDING. VIII. A SINGLE NUMERICAL MEASURE OF THE TOTAL AMOUNT OF INBREEDING[1]

1. In the earlier numbers of these Studies, and particularly in VII,[2] methods have been given for measuring the amount or degree of inbreeding exhibited in a particular pedigree by a series of inbreeding coefficients, Z_1, Z_2, Z_3, . . ., Z_n, one for each ancestral generation. The inbreeding for the whole pedigree is indicated by an inbreeding curve, formed by plotting and connecting by a line the several coefficients.

2. From the earliest stages of this investigation the writer has been aware of the desirability of a *single* numerical measure, to supplement or replace the inbreeding curve as a designation of the total inbreeding exhibited. Such a designation has now been found, which, it is believed, uniquely and rigorously meets the requirements. It is the purpose of the present paper to describe this new constant.

Consider Fig. 1. This gives, in the heavy line and solid circles, the inbreeding curve for 9 ancestral generations of the Brown Swiss bull, Saxton (2668).[3] The values of the inbreeding coefficients are:

$Z_1 = 0$,	$Z_4 = 12.50$,	$Z_7 = 26.95$,
$Z_2 = 0$,	$Z_5 = 17.19$,	$Z_8 = 28.91$,
$Z_3 = 6.25$,	$Z_6 = 21.09$,	$Z_9 = 29.30$.

The smooth curve of Fig. 1 is the inbreeding curve for continued brother × sister mating. This represents the closest or maximum degree of inbreeding possible in sexually reproducing organisms.

It is clear from inspection of this diagram, that Saxton is much less intensely inbred in fact than he would be if in all his ancestry the matings had been of brother × sister out of brother

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station. No. 118.

[2] Pearl, R., AMER. NAT., 1917, in press.

[3] Cf. Pearl, R., these Studies, I. AMER. NAT., Vol. XLVII., p. 603, 1913.

× sister, etc. This is evident, in the first place, because the ordinates of the Saxton curve, *a c* are nowhere as high as those of the brother × sister curve, *a b*. But it would also be equally clear that Saxton was less inbred than the maximum possible amount if the last ordinate at *c*, for example, had a value of 99.6, as does the corresponding ordinate of the maximum curve.

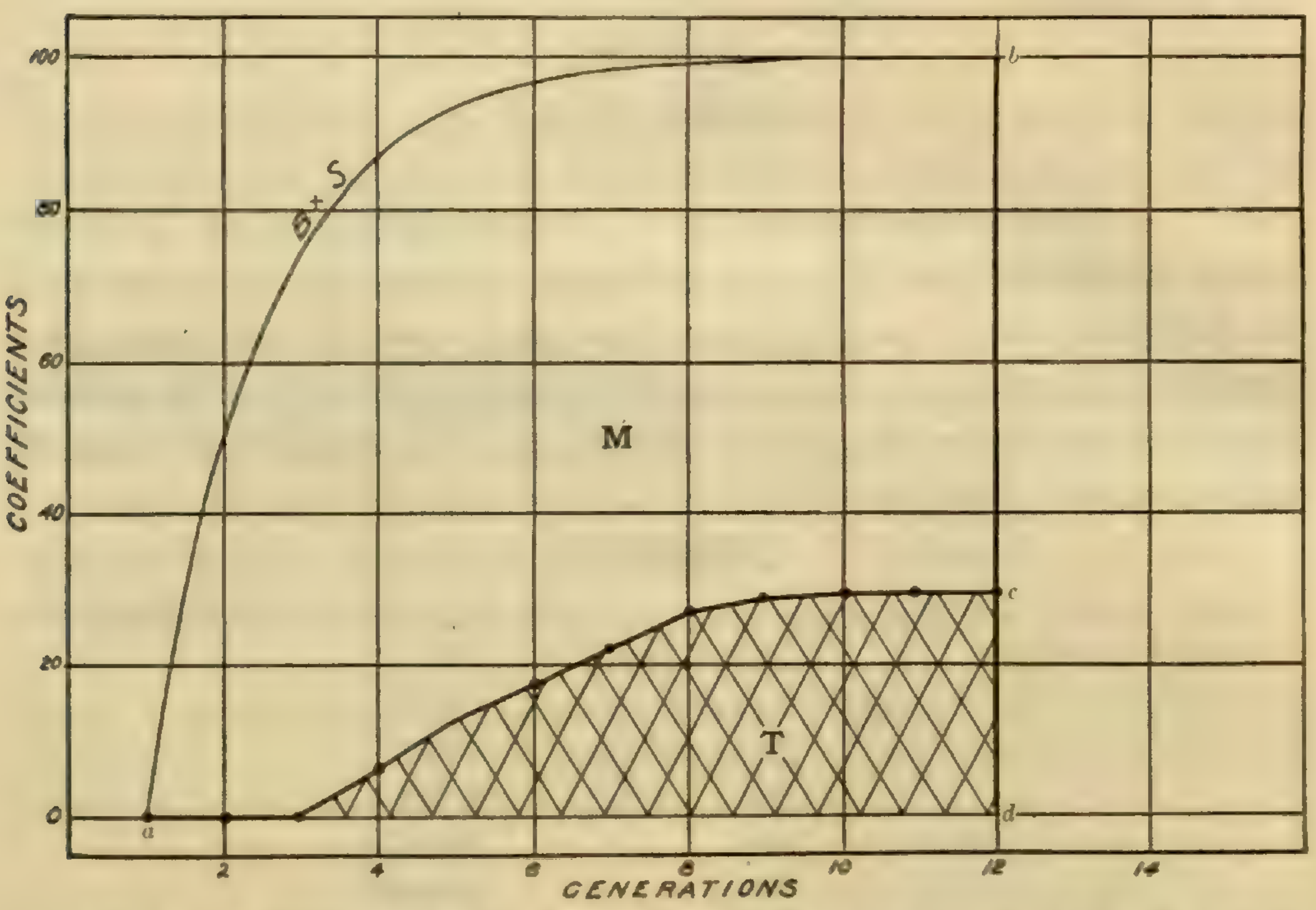


FIG. 1. Diagram showing the inbreeding curve of Saxton, a Brown Swiss bull, in the heavy lines and solid circles. The smooth, light line curve is the curve of maximum inbreeding (continued brother × sister breeding). For further explanation see text.

Upon consideration it appears that the real measure of comparative amount or degree of inbreeding, considering the pedigree as a whole, is given by the *area* included by the particular inbreeding curve under discussion, as compared with the corresponding area of the maximum (brother × sister) curve. Thus in Fig. 1, Saxton is less inbred than the maximum possible amount to an extent proportionate to the amount by which the area *T* (*a c d*) is smaller than the area *M* (*a b d*). This consideration gives us the desired method of uniquely expressing the total amount or degree of inbreeding. It only remains to consider practical methods of calculation.

3. Theoretically one should integrate the maximum (brother × sister) curve, and the observed curve, and compare the areas derived from such integrations. Practically this is not possible, because many observed curves of inbreeding can not be fitted by

any simple, readily integrable theoretical curves. We shall hence be compelled in this case to make use of the expedient so frequently employed in applied mathematical problems of all sorts, namely, to take finite summation as a sufficiently close approximation to integration. Doing so, we may take as the simple expression of total inbreeding, to and including the nth generation, the following:

$$\frac{Z_{T_n}}{100} = \frac{\Sigma_{Z_1}^{Z_n}}{F_{T_n}}, \qquad \text{(i)}$$

where Σ denotes summation of all values between the inclusive limits indicated, and F_{T_n} is a constant having the value set forth in Table I. F_{T_n} is of course the total area of the maximum brother $\times$ sister curve up to and including the $n+1$-th generation. Since these successive values are constant they may be tabled once for all.

TABLE I

VALUES OF F_{T_n}, THE INCLUSIVE AREAS OF THE MAXIMUM INBREEDING CURVE

Ancestral generation	n	F_{T_n}
2	1	50
3	2	125
4	3	212.5
5	4	306.25
6	5	403.125
7	6	501.5625
8	7	600.78125
9	8	700.390625
10	9	800.19531254
11	10	900.09765625
12	11	1000.048828125
13	12	1100.0244140625
14	13	1200.01220703125
15	14	1300.006103515625
16	15	1400.0030517578125

In using the form of total inbreeding coefficients shown in (i) there is one caution which must be carefully observed. This is that only so many generations should be used as to include the one in which the observed Z taken first reaches its highest value for the pedigree under discussion, and not any beyond that one. This will usually be for the earliest ancestral generation of the pedigree, but not always.

4 One would not, of course, use in practical calculation such excessive numbers of decimals as are tabled from this point on.

4. We may now consider some numerical illustrations. Let us take first the bull Saxton, for which the several observed inbreeding coefficients have already been given. We have

$$\Sigma_{Z_1}^{Z_9} = 142.19$$

and hence, from Table I,

$$Z_{T_9} = \frac{14219}{800.1953} = 17.8.$$

Or we may say that Saxton is inbred in ten ancestral generations, taken together 17.8 per cent. of the maximum amount possible in those generations.

For comparison some other figures may be examined. Pearl and Patterson[5] have given mean values of the inbreeding coefficients for four groups of Jersey cattle: (*a*) Random sample bulls, (*b*) register of merit bulls, (*c*) random sample cows, (*d*) register of merit cows. It will be of interest to reexamine these figures by the method here described. The results are given in Table II.

TABLE II

TOTAL INBREEDING COEFFICIENTS FOR JERSEY CATTLE. (PEARL AND PATTERSON DATA)

Group	Total Inbreeding Coefficients Z_{T_7}		
	Lower Limiting Values	Upper Limiting Values	Mean
1. General population (random sample) bulls	25.39	30.48	27.94
2. Register of merit bulls.....	24.52	29.17	31.85
3. General population (random sample) cows	27.46	31.74	29.60
4. Register of merit cows	20.72	27.08	23.90

From this table we see that American Jersey cattle, as judged by random samples of the general population, are about 28 to 30 per cent. as closely inbred as the maximum possible amount, taking account of the first eight ancestral generations *as a whole*.

It is not desirable to go further into the discussion of these Jersey data, since the purpose of this note is simply an exposition of method. This new method makes possible exact and unique numerical comparison between pedigrees in respect of the degree of inbreeding which they exhibit in the same number of ancestral generations.

RAYMOND PEARL

[5] Pearl, R., and Patterson, S. W., *Proc. Nat. Acad. Sci.*, Vol. 2, p. 60, 1916.

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THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG[1]

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I. THE COMPLEXITY OF ORGANIZATION OF THE INSECT EGG

1. *Introduction*

THE morphological and experimental investigations of the germ-cell cycle in insects which the writer has carried on during the past ten years have resulted in the accumulation of many data which indicate the complexity of organization of the eggs of these animals, and suggest hypotheses regarding the nature and genesis of this organization. That the animal egg at the time development begins does not consist of a homogeneous mass of protoplasm, as the old theory of epigenesis required, but is a highly organized cell containing various kinds of protoplasm localized in definite regions has been proved conclusively by numerous investigators working with the eggs of many different species. The degree of organization at the time of fertilization varies according to the species of animal, but all embryologists admit that the insect egg is one of the most highly organized of all.

The nature and genesis of the different kinds of protoplasm in the insect egg is the principal problem discussed in the following pages. This problem is the logical successor of those dealing with cell lineage and the organization of the egg at the time development begins. The re-

[1] Presented to the Johns Hopkins Scientific Association on October 9, 1917.

lations between this problem and the larger problem of heredity and development are very close indeed. The study of heredity is concerned not only with the adult animal, but also with every stage in the development of the adult since the fertilized egg, or embryo, or larva that arises from it, is an individual just as is the fully developed animal. The organization of the fertilized egg is the result of the processes of differentiation that take place at each stage in the history of the egg and sperm from the time the primordial germ cells are segregated until the highly specialized gametes have become fully formed. The period extending from the formation of the primordial germ cells to and through the growth period of the gametes is one of the least known in the entire history of the individual. It is nevertheless a most important period, for during this time, at least in the insect egg, the principal axes of the individual are established and different kinds of cytoplasm are elaborated and localized that are predetermined to form definite parts of the embryo.

The stages in the germ-cell cycle in insects belonging to different orders, families, genera or species are often quite different, as is to be expected, hence the data on which this paper is based were derived from the study of a number of species. On this account it seems best to give an abridged description of the germ-cell cycle in one group and include, wherever necessary, data derived from the study of other groups. The principal work has been carried on with representatives of the orders Diptera, Hymenoptera and Coleoptera, and of these, the order Coleoptera has furnished the best material for experimental purposes. We will select, therefore, the eggs of certain chrysomelid beetles for descriptive purposes.

2. *The Structure of the Insect Egg at the Time of Deposition*[1]

At the time of deposition (Fig. 1) the beetle's egg consists largely of deutoplasm—a substance which is used

[1] Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

up during the growth of the embryo. This deutoplasm is composed of vitelline spheres, which contain refringent granules, the vitelline bodies, and of oil globules. The deutoplasmic bodies are embedded in a viscid cytoplasmic matrix which, however, is very slight in amount as compared with the deutoplasmic material. At the periphery of the egg is a thin cortical layer of cytoplasm which is continuous with the cytoplasm in which the vitelline spheres lie. A short distance back of the anterior pole of the egg is a thickening of this cortical layer in which the maturation divisions of the oocyte nucleus occur. The cytoplasm appears to be homogeneous except at the posterior end, at which place, in many insects, inclusions have been discovered which appear to play a rôle in the formation of the primordial germ cells.[2]

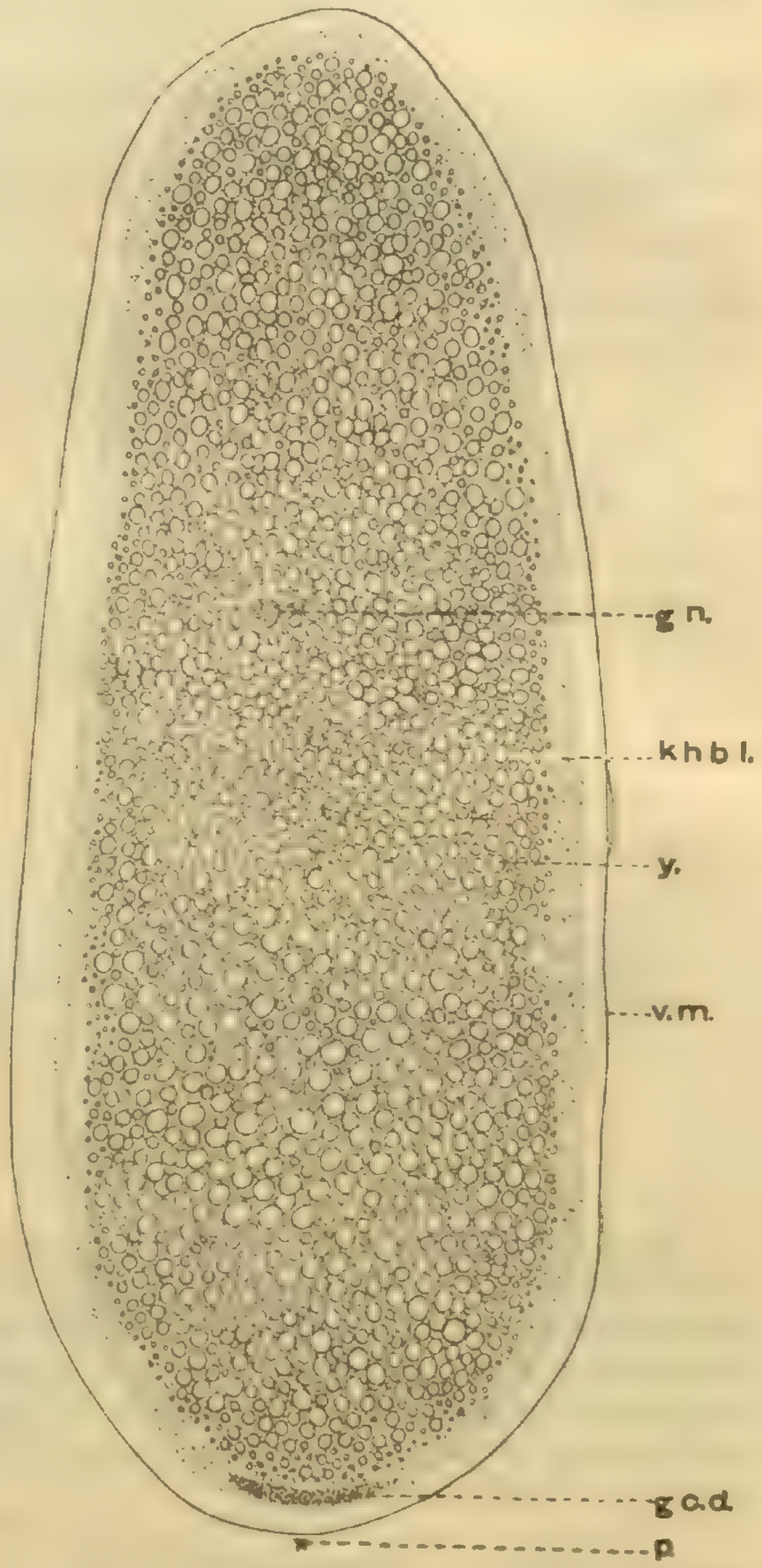

FIG. 1. Longitudinal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd* = germ-cell determinants. *gn* = germ-nuclei copulating. *khbl* = cortical layer of cytoplasm. *p* = posterior end. *vm* = vitelline membrane. *y* = yolk. (From Hegner, 1909.)

These inclusions in the chrysomelid beetles take the form of a polar disc of granules which I have called germ-cell, keimbahn, or germ-line determinants.[3] Similar inclusions (Fig. 2) have been

[2] Hegner, 1909, *Journ. Morph.*, Vol. 20.

[3] Hegner, 1908, *Biol. Bull.*, Vol. 16.

[4] Weisman, 1863, *Zeit. f. wiss. Zool.*, Bd. 13; Metschnikoff, 1866, *ibid.*,

noted in Diptera,[4] in parasitic Hymenoptera,[5] and in Hymenopterous gall flies.[6] There should also be mentioned in this connection minute bodies that have been

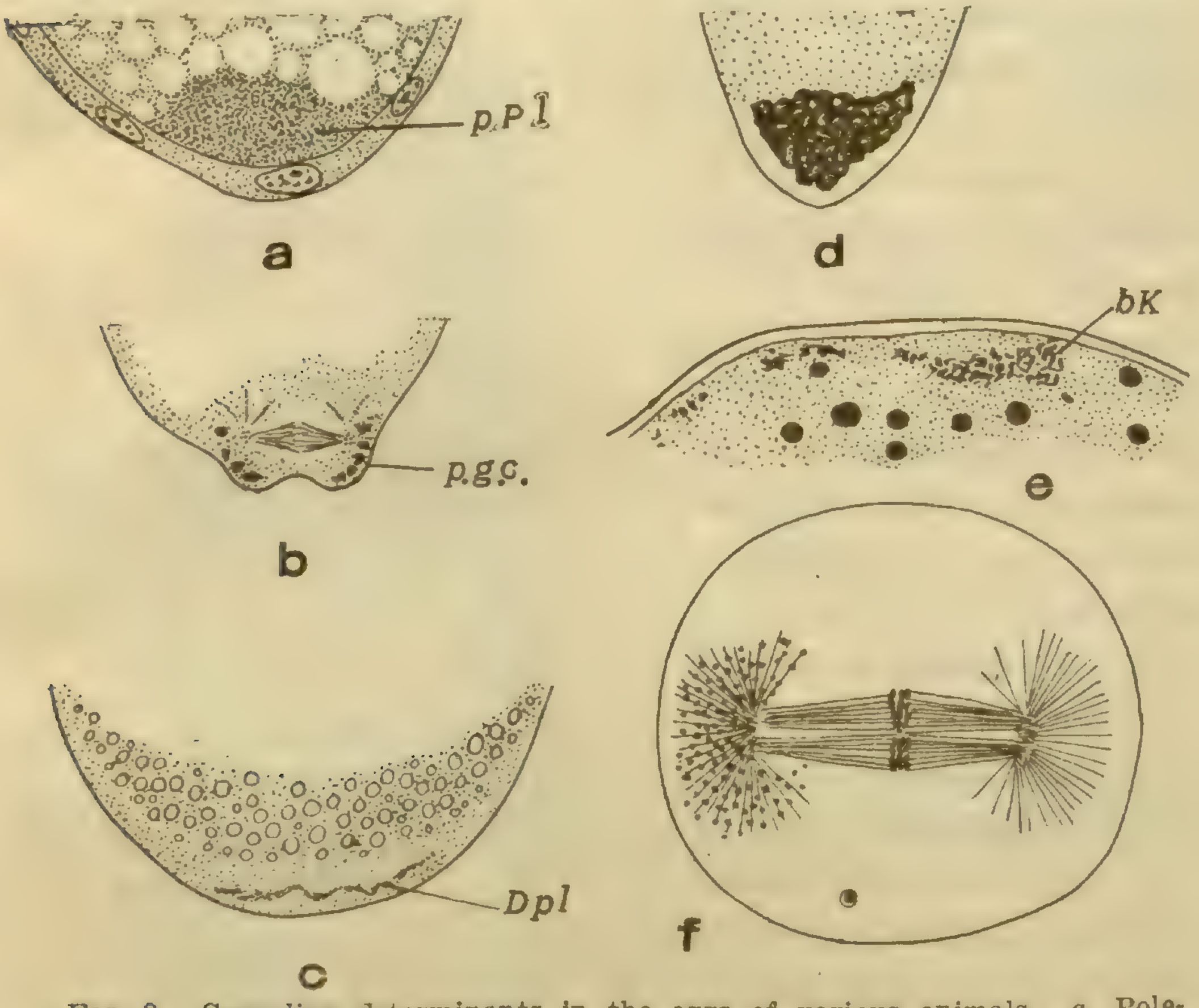

FIG. 2. Germ-line determinants in the eggs of various animals. *a.* Pole-plasm (*p Pl*) at the posterior end of the egg of *Miastor* (Hegner, 1914). *b.* Keimbahnplasma (*pgc*) at the posterior end of the egg of *Chironomus.* One of the four cleavage nuclei in undergoing mitosis. (Hasper, 1911.) *c.* Dotter-platte (*Dpl*) at the posterior end of the egg of *Calliphora.* (Noack, 1901.) *d.* Germ-line determinant at the posterior end of the egg of *Apanteles.* (Hegner, 1915.) *e.* Besondere Körper (*bK*) in the egg of *Sagitta.* (Elpatiewsky, 1909.) *f.* Ectosomen at one end of the first cleavage spindle in the egg of *Cyclops.* (Amma, 1911.)

discovered in the cytoplasm of the eggs of the carpenter ant, *Camponotus,* and in those of various other insects.[7]

Bd. 16; Ritter, 1890, *ibid.,* Bd. 50; Noack, 1901, *ibid.,* Bd. 70; Kahle, 1908, *Zoologica,* Bd. 21; Hasper, 1911, *Zool. Jahrb.,* Bd. 31; Hegner, 1912, *Science,* Vol. 36; Hegner, 1914, *Journ. Morph.,* Vol. 25.

[5] Silvestri, 1906, *Boll. Labor. Zool. R. Sc. Agr. Portici,* Vol. 1; Silvestri, 1908, *ibid.,* Vol. 3; Silvestri, 1914, *Anat. Anz.,* Bd. 47; Silvestri, 1915, *Boll. Labor. Zool. R. Sc. Agr. Portici,* Vol. 10; Silvestri, 1916, *Rend. D. R. Accad. D. Lincei,* Vol. 25; Martin, 1914, *Zeit. f. wiss. Zool.,* Bd. 110; Hegner, 1914, *Anat. Anz.,* Bd. 46; Hegner, 1915, *Journ. Morph.,* Vol. 26.

[6] Hegner, 1915, *Journ. Morph.,* Vol. 26.

[7] Blochmann, 1886, *Festsch. nat.-med. Verein zu Heidelberg;* Sulc, 1906,

These have been considered symbiotic bacteria, but their true nature remains yet to be definitely established.

3. *Cleavage*

The first cleavage nucleus of the beetle's egg lies somewhat anterior of the center in a small island of cytoplasm that is continuous with the cytoplasm that surrounds the deutoplasmic bodies (Fig. 1, *gn*). During early cleavage no cell walls are formed, but after each division the daughter nuclei move a short distance apart and then divide again. Successive divisions and migrations of the cleavage nuclei (Fig. 3) finally result in the production of

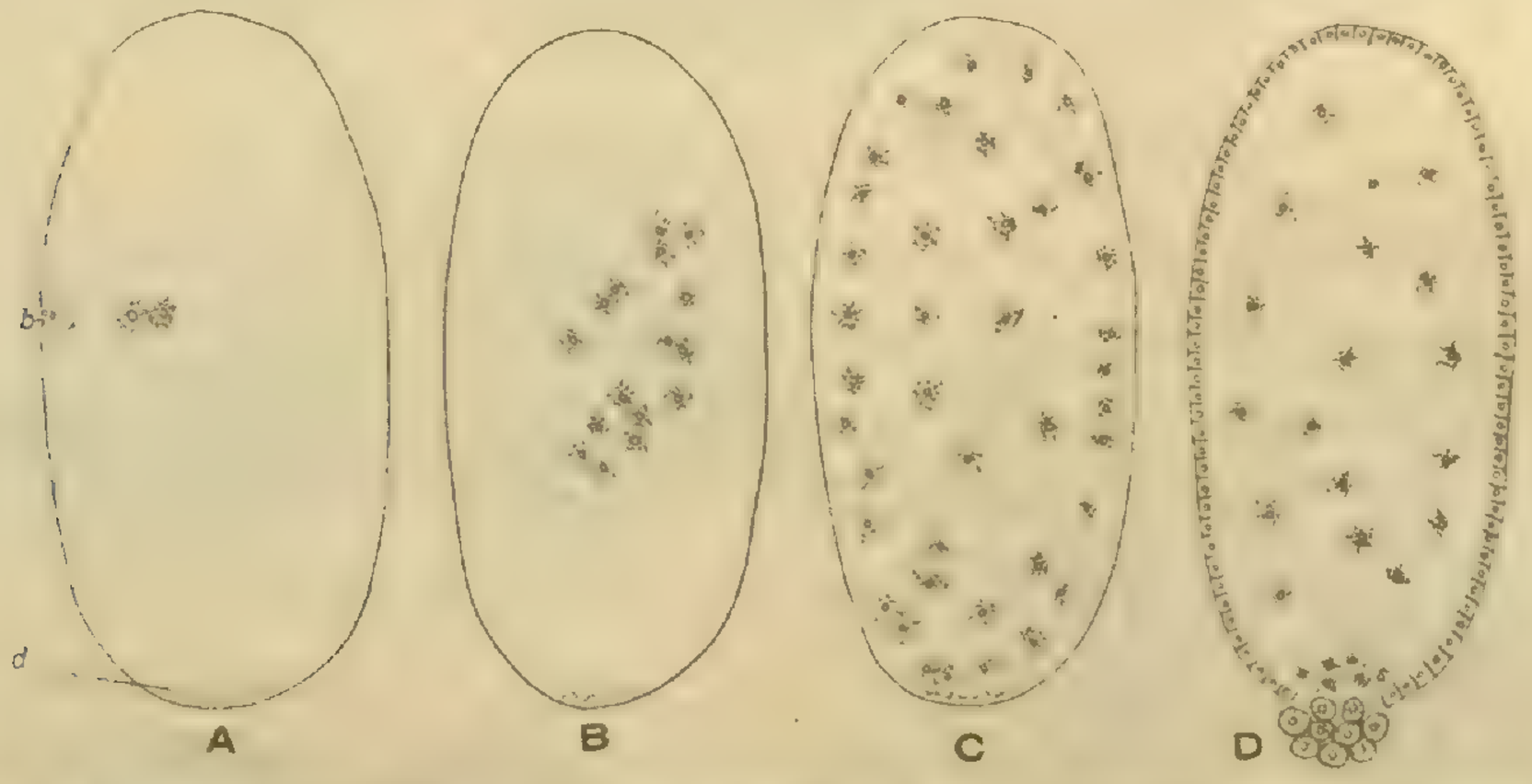

FIG. 3. Diagrams showing four stages in the cleavage and blastoderm formation of the chrysomelid beetle's egg. *a*. Two-cell stage. *b* = polar bodies. *d* = pole disc. *b*. Sixteen-cell stage. *c*. Just before formation of blastoderm. *d*. Blastoderm stage. At the posterior end are the primordial germ cells. (Hegner, 1909, 1914.)

hundreds of nuclei, which come to lie just beneath the cortical layer of cytoplasm, and are each surrounded by an irregular mass of cytoplasm. The fusion of these cytoplasmic masses with the cortical layer then takes place, followed by the intervention of cell walls, thus forming a blastoderm of a single layer of cells, each of which contains a cleavage nucleus, part of the cytoplasm which it brought to the periphery with it, and a portion of the

Stzber. böhm. Gesellsch. Wiss. Prag.; Sulc, 1910, *ibid.;* Mercier, 1907, *Arch. Protistenk.*, Bd. 9; Pierantoni, 1910, *Zool. Anz.*, Bd. 36; Buchner, 1912, *Arch. Protistenk.*, Bd. 26; Tanguary, 1913, *Bull. Ill. St. Lab. Nat. Hist.*, Vol. 9; Hegner, 1915, *Journ. Morph.*, Vol. 26.

cortical layer. Not all of the cleavage nuclei take part in blastoderm formation, many of them remaining behind in the yolk to aid in breaking down this substance.

4. *The Origin of the Primordial Germ Cells*

Blastoderm formation is interrupted at the posterior end of certain chrysomelid beetles' eggs by the segregation of the primordial germ cells (Fig. 4). Those cleav-

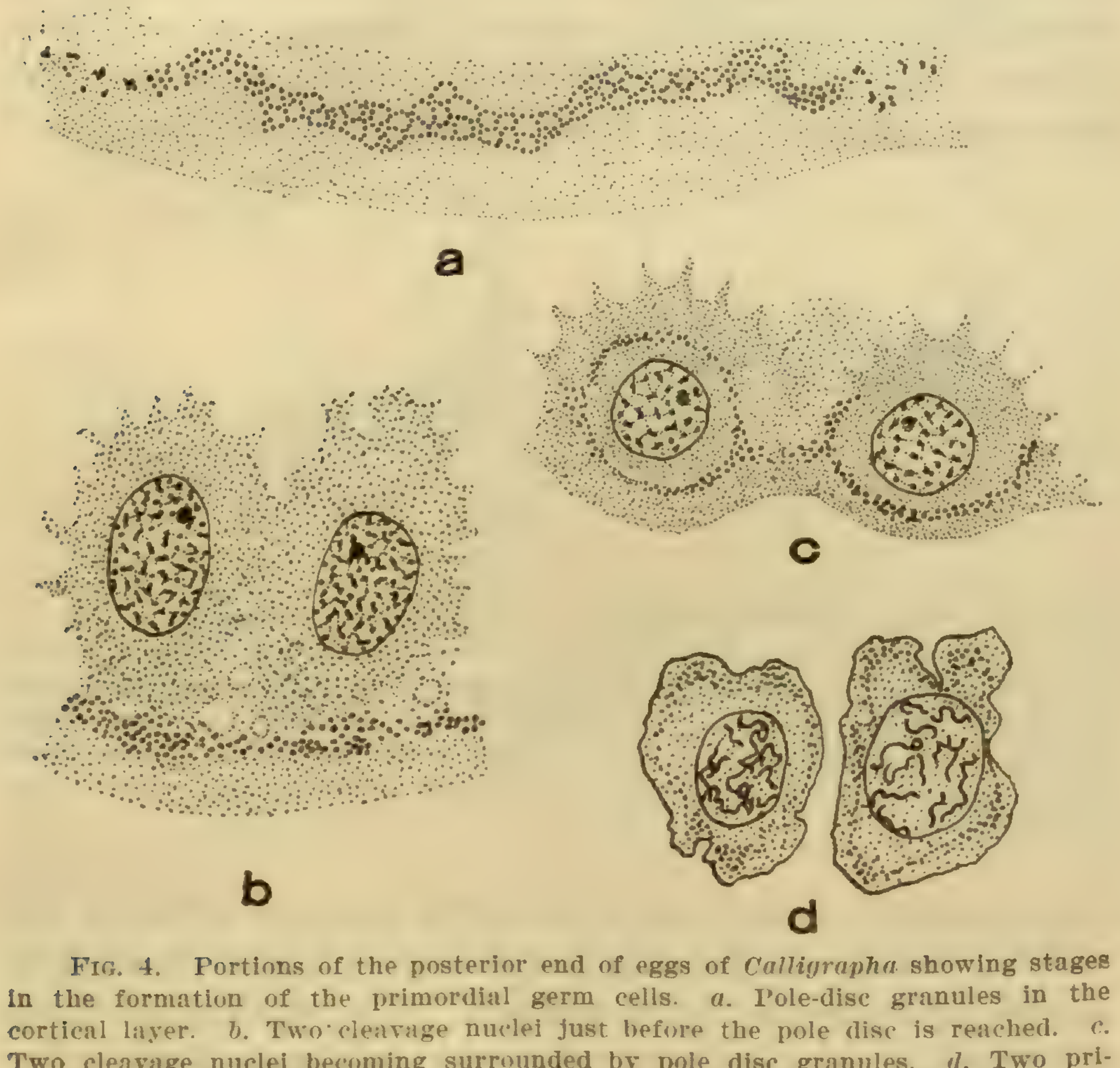

FIG. 4. Portions of the posterior end of eggs of *Calligrapha* showing stages in the formation of the primordial germ cells. *a*. Pole-disc granules in the cortical layer. *b*. Two cleavage nuclei just before the pole disc is reached. *c*. Two cleavage nuclei becoming surrounded by pole disc granules. *d*. Two primordial germ cells entirely separated from the egg. (Hegner, 1909.)

age nuclei that encounter the granules of the pole-disc do not produce blastoderm cells, but continue their migration and are finally cut off from the rest of the egg as distinct cells. These are the primordial germ cells, of which there are sixteen. Each of these cells contains a portion of the cortical layer that includes pole-disc gran-

ules, thus differing in content from the blastoderm cells. They differ also from blastoderm cells in size, being considerably larger. This is probably due at first to the inclusion of the pole-disc granules, but later a greater difference in size is brought about by the failure of the germ cells to divide as rapidly as do the blastoderm cells.

An early origin of the primordial germ cells in a similar manner has been described in a number of insects besides Coleoptera, especially in the midge, *Chironomus*,[8] where these cells are derived from one of the cleavage nuclei at the four-cell stage; in the pedogenetic larva of the fly, *Miastor*,[9] where one of the first eight cleavage nuclei becomes the nucleus of the primordial germ cell, in the fly, *Calliphora*,[10] and in parasitic Hymenoptera.[11]

5. *The Formation of the Ovaries*

In chrysomelid beetles, *Chironomus*, *Miastor* and certain other species of insects, the primordial germ cells undergo a multiplication period shortly after they are formed. This is followed by a period during which they become lodged within the embryo—either by the shifting of the surrounding tissues or by migration or by both these processes. At this time also they become separated into two groups; in chrysomelid beetles each group appears to contain thirty-two germ cells; in *Miastor* each consists of four germ cells. One group becomes located on either side of the embryo and later gives rise to one half of the germ glands. The sex of the individual can be determined by the morphology of the germ glands before the young hatches.

The further history of the germ cells in female insects is in general as follows. From each primitive ovary a number of ovarian tubules arise each containing many germ cells (oogonia) which have undergone a multiplication period. The oogonia finally cease dividing and the

[8] Hasper, 1911, *Zool. Jahrb.*, Bd. 31.

[9] Kahle, 1908, *Zoologica*, Bd. 21; Hegner, 1914, *Journ. Morph.*, Vol. 25.

[10] Noack, 1901, *Zeit. f. wiss. Zool.*, Bd. 70.

[11] Silvestri, *l. c.*

ultimate oogonia are ready to enter upon the growth period. A period of differentiation may or may not intervene, according to the species, during which nurse cells are formed. When the oocytes have reached their full size they separate from the ovarian tubule, pass down the oviduct into the vagina and are deposited. Each egg is surrounded by two membranes; a thin inner vitelline membrane and a thicker, outer membrane, the chorion.

6. *The Complexity of Organization of the Insect Egg.*

(*a*) *Comparison between Eggs of Insects and Those of Other Animals.*—Insect eggs differ greatly from those usually employed for the study of egg organization, since they are, as a rule, laid in the air and not in the water, and because cleavage is of the superficial type, cell walls being absent until a comparatively late cleavage stage. The eggs of chrysomelid beetles are particularly favorable for study, since they may be subjected to the most violent experimental conditions without preventing their development.[12]

In insect eggs the character of the blastoderm cells depends, as in holoblastic eggs, upon the kinds of protoplasm they contain, but all those phenomena connected with the position of the cleavage spindle, which have been so carefully studied in the eggs of mollusks, worms, ascidians and other animals, can have no influence upon the localization of different substances in various parts of an insect egg, because in the latter the volume of the egg is thousands of times greater than that of the cleavage spindle. Furthermore in holoblastic eggs differentiated substances are segregated in different cells during early cleavage and are there isolated by cell walls, and to this isolation is attributed in large part the progress of differentiation; but in the insect egg the different kinds of cytoplasm are in direct continuity until hundreds of cleavage nuclei are present, and are not separated by cell walls until the blastoderm is fully formed.

[12] Hegner, 1908, *Biol. Bull.*, Vol. 16; 1909, *Journ. Exp. Zool.*, Vol. 6; 1911, *Biol. Bull.*, Vol. 20.

(*b*) *Results of Experiments with Gravity and Centrifugal Force.*—A chrysomelid beetle, such as *Calligrapha* or *Leptinotarsa,* during the process of egg-laying clings to the under side of a leaf and the end of the egg that emerges first is glued to the leaf by a viscid secretion. Then the egg is pushed back away from the abdomen and another is laid[13] (Fig. 5). In this way from four to

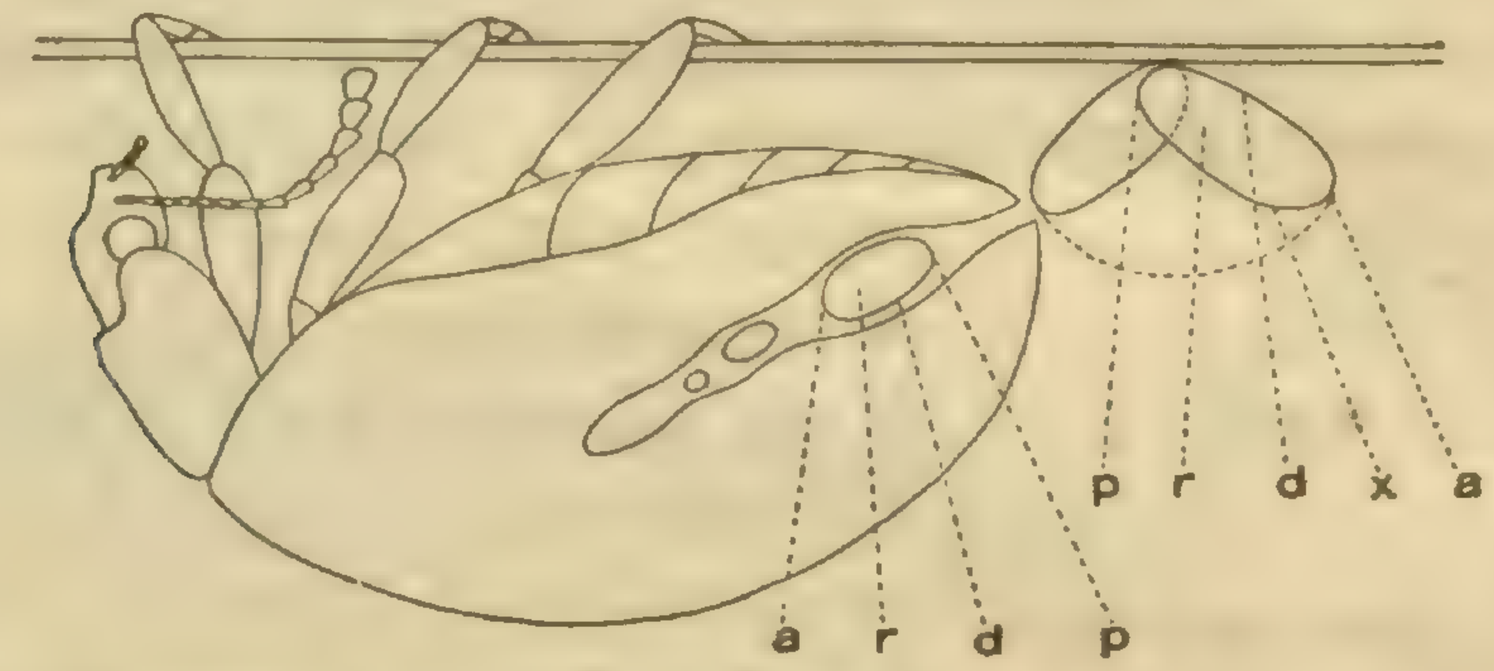

FIG. 5. Diagram showing a chrysomelid beetle, *Calligrapha bigsbyana,* clinging to the underside of a willow leaf and laying her eggs. The relation between the orientation of the egg before and after deposition is indicated by the letters. *a* = anterior. *d* = dorsal. *l* = left. *p* = posterior. *r* = right. *x* = anterior ventral surface where a spot of India ink was placed as a guide for orienting the eggs during experiments. (Hegner, 1909.)

eighty eggs are laid in one group within a period of about an hour. These eggs hatch in approximately five days. A few hours before they hatch the young can be seen distinctly through the semi-transparent egg shell. An examination of hundreds of eggs at this stage in their development has established the fact that the posterior end of the egg is attached to the leaf and the anterior end is free. In every other respect the orientation of the young in the egg corresponds to that of the egg as it lay within the body of the mother before deposition; that is, the ends and various surfaces of the egg are definitely determined before deposition and correspond to the orientation of the mother as indicated in the diagram (Fig. 5). This rigid correspondence between the orientation of the egg and that of the adult is known as the "law of orientation" which was first discovered by Hallez in 1886.

Since the eggs of these beetles are usually attached to

[13] Hegner, 1909, *Journ. Exp. Zool.,* Vol. 6.

the underside of leaves, it was suggested that the orientation of the young might depend upon the force of gravity, but eggs that were first marked with India ink and then placed in every conceivable position with respect to gravity proceeded to develop as though undisturbed.[14] It seemed from this, therefore, that the position of the young must be predetermined in the undeveloped egg.

Several kinds of experiments were performed in order to discover the complexity and fixity of this apparent organization. First, the eggs were subjected to a force greater than gravity by means of a centrifugal machine. Hundreds of eggs were revolved at different rates of speed for various lengths of time and in many different positions. A description of one experiment will serve to illustrate the results obtained.[15] In this experiment freshly laid eggs were placed in cavities in a block of paraffin with the posterior end toward the center of rotation, and were revolved in a hydraulic centrifuge for sixteen hours. The heavier substances were thrown to the outer end and the lighter protoplasm accumulated at the inner end, where an embryo developed (Fig. 6). It is perfectly evident that the protoplasm from the various parts of the egg has, in its new position, developed into the tissue that it would have given rise to if it had been left undisturbed. Normally the yolk would be surrounded by the embryonic tissue and would be en-

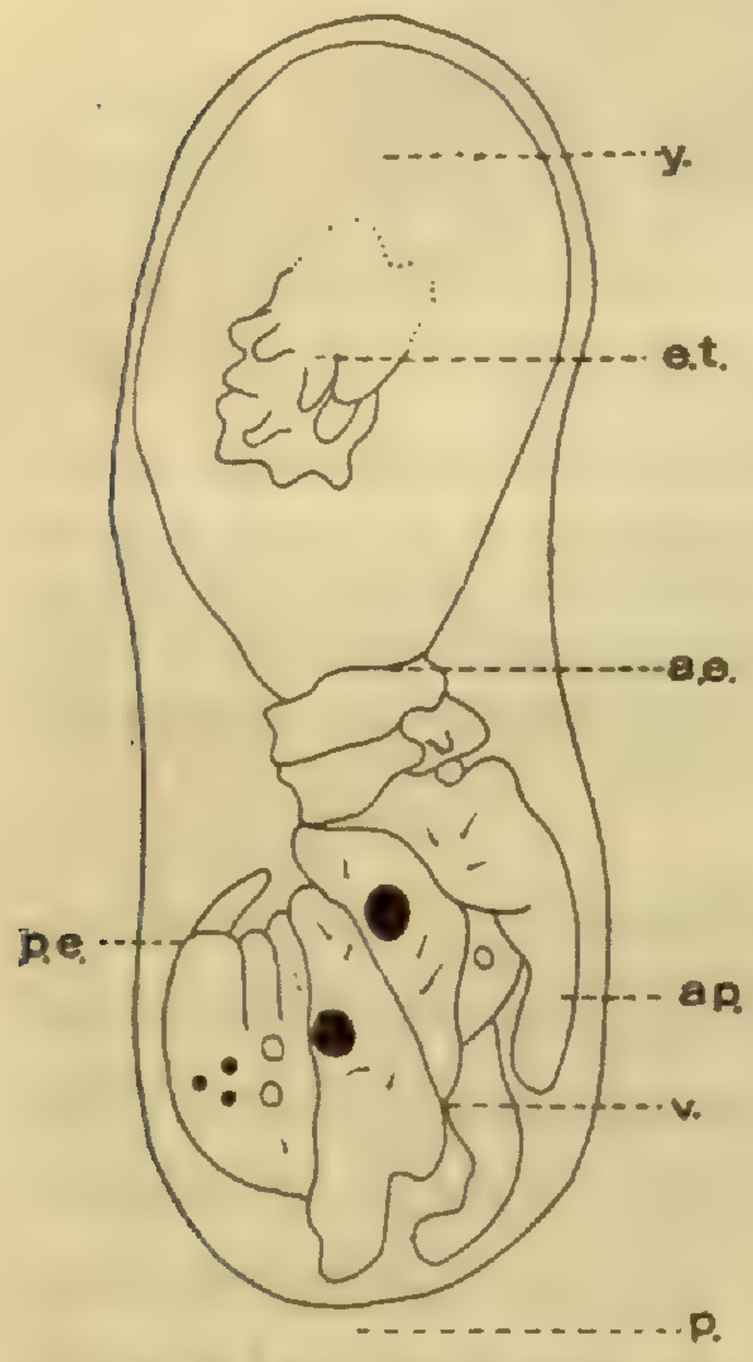

FIG. 6. Side view of a freshly laid egg of *Calligrapha multipunctata,* which was centrifuged for sixteen hours and then taken out and allowed to develop for nine days. *ae* = anterior end of embryo. *ap* = appendage of thorax. *et* = embryonic tissue. *p* = posterior end of egg. *pe* = posterior end of embryo. *v* = ventral. *y* = yolk. (Hegner, 1909.)

[14] Wheeler, 1889, *Journ. Morph.*, Vol. 3; Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

[15] Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

closed by the mid-intestine, but in this case a dwarf embryo has developed without growing around the nutritive material.

The effects of centrifugal force upon insect eggs are different from those produced upon the other types of eggs that have been employed for such experiments. In the eggs of worms,[16] mollusks,[17] etc., apparently the materials that undergo stratification under the influence of centrifugal force have no influence upon the "ground substance" which is "the seat of polarity and pattern of organization of the cell." In the insect egg, the organized protoplasm is almost entirely limited to the cortical layer and this layer may be shifted away from the periphery by a sufficient force and may become massed at the inner light end when an undeveloped egg is centrifuged.

Since the cytoplasm develops in its new situation and proceeds to build up an embryo as nearly normal as is possible under the conditions imposed upon it, it is evident that the potencies of the cytoplasmic areas are predetermined at the time the egg is laid.

It was hoped by means of these experiments with centrifugal force to throw the pole-disc granules and the cytoplasm containing them into some other part of the egg. If the germ cells arose from this material in its new position the conclusion would have been convincing that these substances were necessary for the formation of the reproductive cells. Unfortunately, although the cortical layer at the posterior end was shifted by the centrifugal force, it was impossible to locate accurately the germ cells in the embryos that developed from the eggs that were thus operated upon.

(*c*) *Relation between Cleavage Nuclei and Egg Organization.*—During the course of my early studies of chrysomelid eggs it occurred to me that the nuclei that result from early cleavage might be definite in number and in

[16] Lillie, F. R., 1906, *Journ. Exp. Zool.*, Vol. 3.
[17] Conklin, 1917, *Journ. Exp. Zool.*, Vol. 22.

distribution and that they might be qualitatively different. If this were true, then nuclei of one sort might always migrate into one part of the egg and might determine the nature of the tissue that developed there, and nuclei of other sorts might likewise become located in other predetermined parts of the egg. Careful studies of the origin and migration of the cleavage nuclei[18] have led to the conclusion that the distribution of these nuclei is adventitious and that they are all potentially alike—that is are totipotent—a view that is now held by most embryologists regarding the relative importance of nucleus and cytoplasm during cleavage. That the nuclei may play a part in the differentiation of the cortical layer of cytoplasm during the cleavage period is highly improbable, since definite cytoplasmic organization already exists before cleavage begins. The factors brought into the egg by the spermatozoon, however, have an opportunity at this time to modify the initial organization and thus the early embryo may exhibit paternal characteristics. Whether or not such an influence is exerted at this time is not known.

The kind of tissue that develops from any part of the egg, therefore, depends upon the kind of cytoplasm encountered by the nuclei.

(*d*) *Complexity of Organization as Indicated by the Development of Parts of Eggs.*—More convincing evidence of the presence of a complex and fixed organization in the cytoplasm was derived from operations performed upon eggs with a hot needle. Parts of the freshly deposited eggs were killed by being touched with a hot needle and these parts were thus prevented from taking part in development. The living portions of the eggs continued to develop and in every case produced those parts of the embryo that they would have formed if the egg had not been injured.[19] This seems to prove that every part of the egg cytoplasm is set aside for the pro-

[18] Hegner, 1914, *Journ. Morph.*, Vol. 25.

[19] Hegner, 1911, *Biol. Bull.*, Vol. 20.

duction of a definite part of the embryo, and hence of the larva and adult, and that the cytoplasm is therefore highly organized at the time the egg is fertilized. After such experiments there is no regeneration of substances.

As stated above, the cortical layer of cytoplasm is visibly alike throughout except at the posterior end, where it has embedded in it the pole-disc granules. One of the most interesting results of the operations performed with the hot needle was obtained by killing the posterior portion of the egg containing the pole-disc (Fig. 7). Eggs

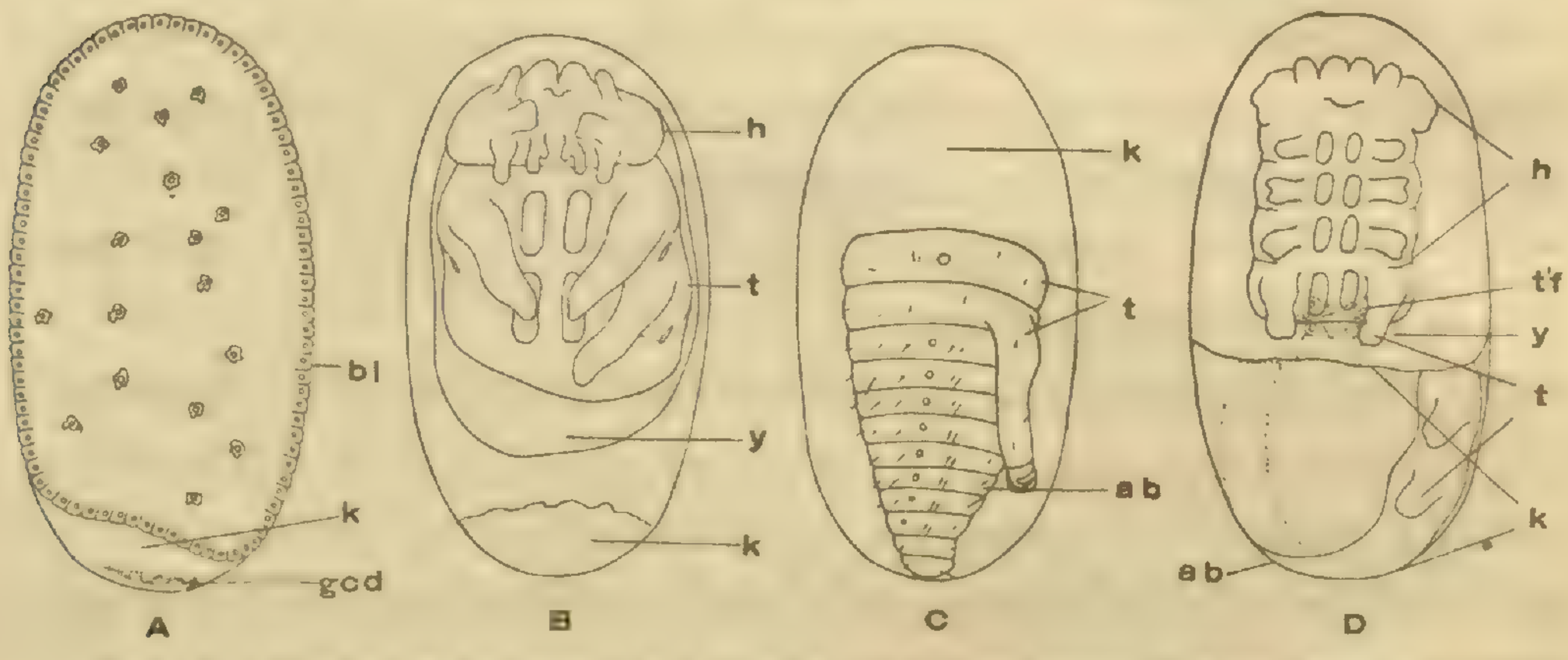

FIG. 7. Diagrams showing the results of killing parts of the eggs of *Leptinotarsa decemlineata* with a hot needle. (Hegner, 1911.)

a. Longitudinal section through an egg twenty-four hours old. The posterior end (*k*) was killed just after the egg was deposited (conditions as in Fig. 1). No germ cells were produced.

b. Ventral view of an embryo three days old. The posterior end (*k*) was killed just after the egg was deposited. The part that remained alive gave rise to the head and about half of the thorax.

c. Side view of an egg five days old. The anterior end (*k*) was killed in the blastoderm stage (Fig. 3, *d*). The part that remained alive produced the abdomen (*ab*) and about half of the thorax (*t*).

d. Ventral view of an egg three days old. The posterior end (*k*) was killed when the embryo was two days old. The anterior half continued to develop. The independence of the tissues is indicated by the minute end of the tail-fold (*tf*) which developed normally after being separated from the rest of the embryo. The two parts of the egg underwent a revolution of ninety degrees during the twenty-four hours succeeding the experiment.

ab = abdomen. *bl* = blastoderm. *gcd* = pole-disc. *h* = head. *k* = portion of egg killed with hot needle. *t* = thorax. *tf* = tail fold. *y* = yolk.

thus modified produced embryos without germ cells, proving that this cytoplasmic region is necessary for their formation. The castration of the individual may also be performed in a similar fashion after the germ cells have been extruded from the egg (Fig. 3, *d*), and it is interesting to note that sexless chicks have recently been pro-

duced in a similar fashion, by removing the region of the embryo from which the germ cells arise.[20]

The blastoderm is of course definitely organized, since its cells contain organized cytoplasm, and by killing parts of the eggs in the blastoderm and later stages results were obtained similar to those produced when fresh eggs were operated upon (Fig. 7, *b*, *c*, *d*).

7. *Summary of Part I*

Summarizing the data briefly given above, we may say:

1. Morphological and experimental studies have proved that the eggs of animals are more or less highly organized at the time of fertilization.

2. We know almost nothing about the nature and genesis of this organization.

3. Descriptions are given of the condition of the eggs of certain chrysomelid beetles at the time of deposition, of the stages of cleavage and blastoderm formation, of the origin of the germ cells, and of the principal stages in the germ-cell cycle.

4. The eggs of certain chrysomelid beetles and of other insects are definitely organized when deposited as indicated by observations on normally developing eggs and by experiments with gravity.

5. This organization exists in the cytoplasm as indicated by a morphological study of cleavage, by experiments with gravity and centrifugal force, and by killing with a hot needle parts of eggs in various stages of development.

6. These observations and experiments prove also that the nuclei up to the time of blastoderm formation are totipotent.

II. THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG

1. *Introduction.*

I have decided to consider the organization of the egg only in this discussion, since it contains everything neces-

[20] Reagan, 1916, Abstracts 14 annual meeting Amer. Soc. Zool.

sary for the production of a complete organism. In many species of insects and other animals, parthenogenesis is a normal phenomenon and in many species whose eggs must ordinarily be fertilized development may be initiated by artificial means.[21] Among these are the eggs of the silkworm moth. It also seems certain that the cytoplasmic regions of the insect egg have reached a high state of morphological and physiological differentiation before fertilization, judging from the results outlined in Part I. of this paper. The cleavage nuclei may possibly exert an influence upon the cortical layer of cytoplasm before the blastoderm is formed, thereby enabling the paternal contribution to the zygote to act, but besides being very improbable, such a phenomenon would, of course, follow rather than precede the establishment of the advanced state of organization that exists in the undeveloped egg.

2. *Constitution of the Primordial Germ Cells*

In certain beetles, flies and parasitic Hymenoptera, the primordial germ cells are visibly different from the rest of the embryonic cells that arise at about the same time. This difference is primarily due to the inclusion within them of visible substances that are located in the egg material from which they originate. The eggs of these different insects are similar in certain respects and different in others. In every instance, however, this visible substance, which forms the germ-line determinants, is situated near the posterior end of the egg, and it is at this point that the primordial germ cells are formed. The origin of the germ-line determinants is not definitely known in any insect, but their position in the egg and their granular appearance are constant.

It has often been pointed out that the primordial germ cells remain in a comparatively undifferentiated state until the individual in which they lie has almost reached maturity, and that they then undergo changes during

[21] Loeb, 1913, "Artificial Parthenogenesis and Fertilization," Chicago.

which they reach a high state of specialization. The discovery of axial gradients of metabolism in the eggs of certain animals in an anterior-posterior direction[22] suggests that this may also be true of insect eggs. If such gradients exist in insect eggs and if the metabolic activity decreases gradually from the anterior to the posterior end, then the primordial germ cells, which arise at the extreme posterior end, are actually the least active metabolically of all the cells of the embryo. Their early separation from the egg would also tend to keep them in an undifferentiated condition since they are on this account less likely to be influenced by the rest of the embryo.

The primordial germ cells in these insect eggs are thus visibly different because of the presence of germ-line determinants and are probably physiologically different, at least in part, because of their position at the posterior end of the egg.

The contents of these cells are as follows (Fig. 4): (1) part of the cortical layer of cytoplasm, (2) part of the cytoplasm which surrounds the cleavage nuclei and which is collected from among the yolk globules, (3) part of the germ-line determinants, and (4) a nucleus with the full amount of chromatin. The fourth item is mentioned because in *Miastor* all of the nuclei that form somatic cells undergo a diminution process, being similar in this respect to *Ascaris*. This chromatin is in *Miastor* entirely maternal since the eggs of this fly that have been studied, develop parthenogenetically.

Nothing very definite has been discovered regarding the arrangement of these substances in the germ cells. The nucleus lies near the center in all of them; the two kinds of cytoplasm soon become indistinguishable; and the germ-line determinants may, at first, be more or less evenly distributed throughout the cytoplasm, as in chrysomelid beetles and *Miastor*, or may be clumped in various parts of the cell, as in *Chironomus*. In every case, however, the germ-line determinants evidently become

[22] Child, 1916, *Biol. Bull.*, Vol. 30.

more or less evenly scattered since they cannot be distinguished in later stages in the germ-cell cycle.

3. *Differential Divisions during the Formation of Nurse Cells*

There is no evidence of any definite localization of substances or physiological processes in the primordial germ cells when formed, nor do these cells exhibit recognizable polarity or symmetry of any kind. As described in preceding pages, they multiply; migrate into or are enveloped by the tissues of the embryo; separate into two groups from which the ovaries on either side of the body arise; and then pass through another period of multiplication. This brings them to the stage just preceding the growth period. At this time phenomena occur in the ovaries of certain species of insects that have a direct bearing upon our problem; these are concerned principally with the differentiation of oocytes and nurse cells. In *Miastor* the nurse cells are mesodermal in origin, and a group of nurse cells and one oocyte become enclosed within a sheath of epithelial cells (Fig. 8). As the oocyte increases in size it elongates, and then for the first time in its history exhibits recognizable polarity; the anterior end adjoining the group of nurse cells. Polarity may, however, have been present from the time the primordial germ cell was first formed, corresponding to that of the parental egg. The germinal vesicle soon becomes eccentric, but whether or not this indicates that bilateral symmetry has also been

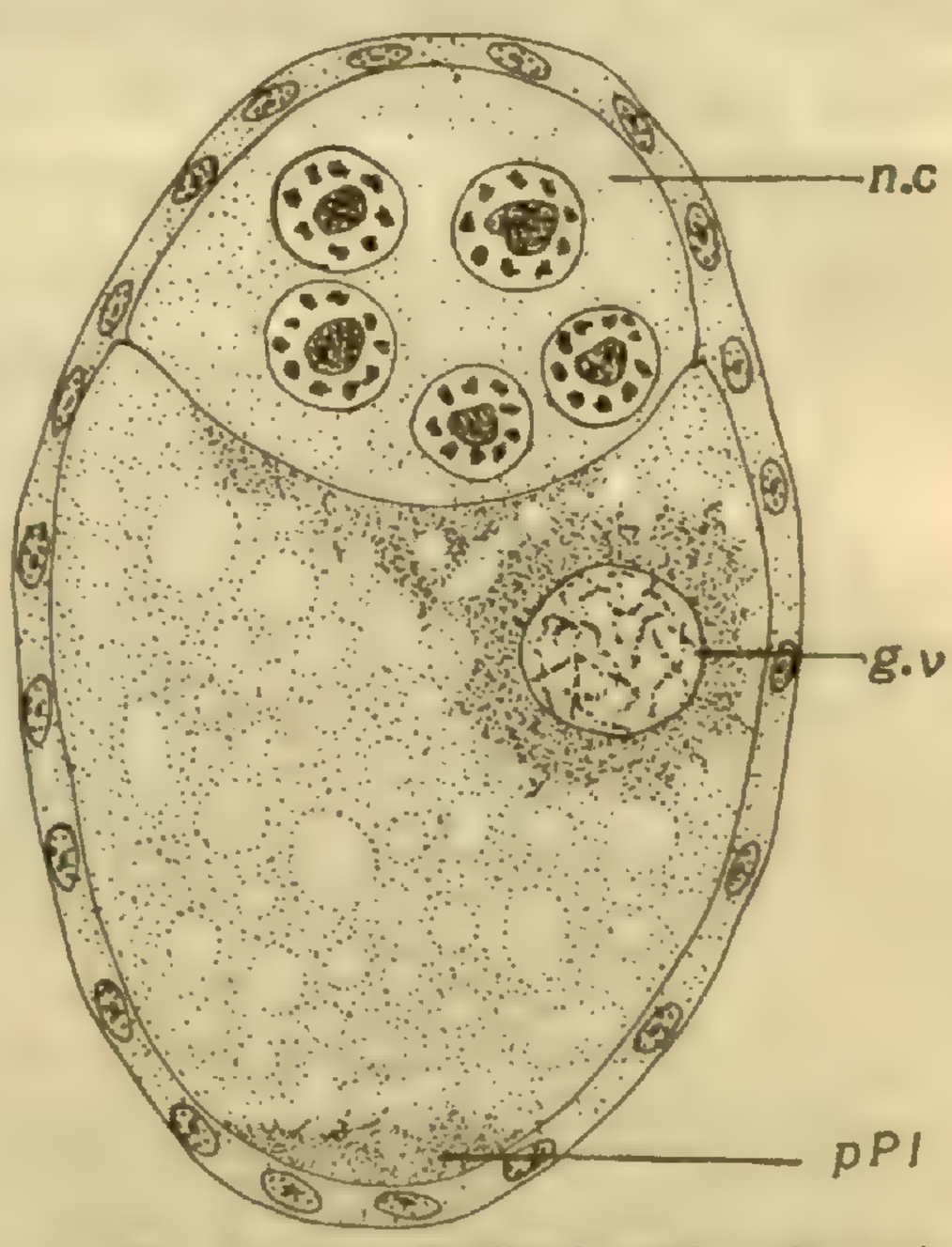

FIG. 8. Longitudinal section through a young oocyte of *Miaster* and its accompanying nurse cells (*nc*). *gv* = germinal vesicle. (Hegner, 1914.)

determined, as it does in certain other insects, is unknown. It is thus certain that polarity exists soon after the beginning of the growth period and that bilaterality is probably also established at an early stage.

The differentiation of oocytes and nurse cells in dytiscid and gyrinid beetles is of peculiar interest, although the early and later history of the germ cells in these insects is not known. In the diving beetle, *Dytiscus marginalis*,[23] a single oogonium gives rise to fifteen nurse cells and one oocyte. The oocyte and its mother cell, grandmother cell, and great-grandmother cell can be dis-

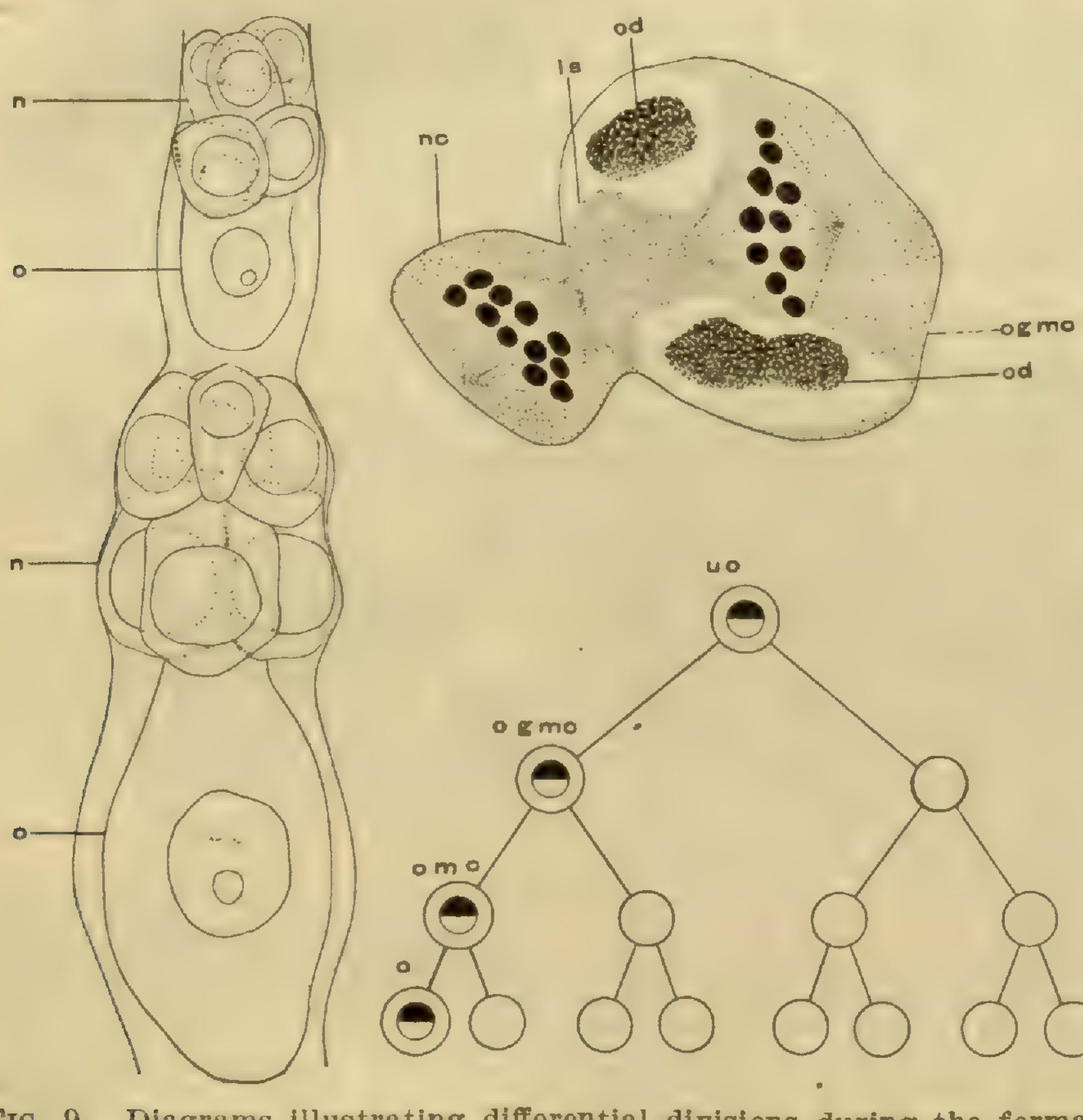

FIG. 9. Diagrams illustrating differential divisions during the formation of nurse cells in the whirligig beetle, *Dineutes nigrior*. (Hegner and Russell, 1916.)

a. Part of the growth zone of an ovarian tubule showing two oocytes (*o*) each accompanied by seven nurse cells (*n*).

b. Division of two-cell stage. An ultimate oogonium has divided forming a nurse cell (*nc*) and an oocyte grandmother cell (*ogmc*) containing the oocyte determinant. *is* = intercellular strand.

c. Diagrammatic representation of oocyte differentiation. The plain circles indicate nurse cells. *uo* = ultimate oogonium containing the oocyte determinant within its nucleus. *ogmc* = oocyte grandmother cell. *o* = oocyte.

[23] Giardina, 1901, *Internat. Monatssch. f. Anat. u. Phys.*, Bd. 18; Debaisieux, 1909, *La Cellule*, T. 25; Gunthert, 1910, *Zool. Jahrb.*, Bd. 30.

tinguished from the nurse cells by the presence of a peculiar ring of nuclear material within the cytoplasm and by their larger size. The gyrinid beetle, *Dineutes nigrior* (Fig. 9), resembles *Dytiscus* in general, but the ultimate oogonium passes through one less division, thus giving rise to one oocyte and only seven nurse cells.[24] The important fact is that during these differential divisions, in both cases, the nurse cells, which may be considered somatic since they are unable to reproduce, are deprived of part of their nuclear material. Apparently they differ from their sister cell, the oocyte, in this one respect, and it is therefore the presence of this nuclear material that makes it possible for the oocyte to develop into a new individual. This is one of the most striking cases of the passage of large masses of nuclear material into the cytoplasm. No such differential divisions have been discovered in chrysomelid beetles nor in the other insects where the nurse cells arise from oogonia, but they may occur in some way that has not been revealed by our methods of research.

The writer has discussed this subject rather fully with relation to the origin of nurse cells and oocytes in the honeybee.[25] In this insect a single oogonium gives rise to a rosette-like group of cells that are connected with one another by strands—probably of a mitochondrial nature—the remains of preceding mitotic divisions. There is no visible difference among the cells in a rosette which are hence apparently potentially alike. Nevertheless one or several from each rosette enlarge to form oocytes which are nourished by the rest acting as nurse cells. What determines the differentiation of certain cells into oocytes is not known but the following hypotheses have been expressed.

Three explanations have occurred to me: (1) There may be differential changes during the mitotic divisions in rosette formation as in *Dytiscus* resulting in one or more cells (oocytes) which differ in con-

[24] Hegner and Russell, 1916, *Proc. Nat. Acad. Sc.*, Vol. 2.

[25] Hegner, 1915, *Journ. Morph.*, Vol. 26.

tent from the others (nurse cells). No visible changes of this sort were observed. (2) The polarity of the rosettes may influence the cells in such a way that those near the center of the ovariole and closest to the zone of differentiation tend to develop into oocytes. (3) Those cells of the rosettes which reach the zone of differentiation first are stimulated to become oocytes and by their growth and differentiation prevent the other cells of the rosettes from similar changes.

4. *Constitution of the Oocyte at the Beginning of the Growth Period*

Very soon after the nurse cells are formed and the oocytes begin to enlarge the main axis of the oocyte in all

FIG. 10. The formation of oocytes in the honeybee. (Hegner, 1915.)
a. Part of an ovariole showing the rosettes (*r*) each resulting from the division of a single oogonium.
b. Part of an ovariole in the zone of differentiation showing five oocytes (*o*), many nurse cells (*n*) and epithelial cells (*e*).

insects seems to be established. The germinal vesicle at the same time changes its position from the center of the cell to a point near the nurse-cell chamber at the anterior end as described above in *Miastor* (Fig. 8). At what stage bilateral symmetry becomes fixed has not been determined.

The oocytes of insects at the beginning of the growth

period differ from the other cells in the body in the following ways. (1) In all cases where germ-line determinants occur the oocytes alone are provided with them and with the cytoplasm in which they are embedded. (2) In insects like *Miastor* a full amount of chromatin is present only in the oocytes. (3) In *Dytiscus*, *Dineutes* and probably other insects the oocytes contain nuclear material of which the nurse cells are deprived, but this may be interpreted simply as a means of inhibiting the reproduction of the latter and of changing them into nurse cells. (4) The oocytes seem to have no influence upon the development of the individual in which they lie, as indicated by castration and transplantation experiments,[26] and are in a comparatively undifferentiated condition when the growth period begins.

[26] Meisenheimer, 1912, Fest. 60 Geburtstage von Dr. J. W. Spengel III.; Kopec, 1911, *Arch. Entev.-mech.*, Bd. 33.

(*To be continued*)

INHERITANCE OF FERTILITY IN SOUTHDOWN SHEEP[1]

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INTRODUCTION

YOUATT (14)[2] says:

The disposition to twinning is undoubtedly hereditary:

> "Ewes yearly by lambing rich masters do make:
> The lambs of such twinners for breeders go take."

Flockmasters of the last century have made selections on this assumption, while the increased number at a birth in the progeny of ewes born multiparously as compared to the progeny of those born singly has been demonstrated by several investigators. In general there has been shown to be an increase in number produced at a birth as the average birth values of the animals lambing increase. Thus, Rietz and Roberts (13) present the following in Shropshires, the number at a birth being represented by the figures 1, 2, or 3:

Sire	Dam	Offspring	No. Cases
1.....	1	1.3452±0.0059	3,059
2.....	1	1.3946±0.0073	2,088
1.....	2	1.4171±0.0067	2,436
2.....	2	1.4548±0.0088	1,550
One parent a triplet		1.6076±0.030	158

Experimental investigations of the inheritance of twinning in sheep have been attempted in few cases. Ains-

[1] Paper No. 5 from the Laboratory of Animal Technology, Kansas Experiment Station.

[2] Reference is made by number to literature cited at close of paper.

worth-Davis and Turner (1) reported a preliminary investigation on this subject, but their numbers are too small and results too contradictory, as published, even to be indicative of the method of inheritance. Arkell and Jones (7) at the New Hampshire station also instituted investigations along this line, but have published no results.

Due to the environmental and physiological factors involved in multiple births, as well as to the economic impracticability of maintaining large flocks under rigid experimental conditions, there are at hand no considerable masses of experimental data which yield evidence on this point, nor are the probabilities great that such experiments will ever be conducted on an adequate scale; hence the bulk of evidence on the inheritance of fertility must come from breeders' flocks or from breed registry records.

The Fertility Problem

High fertility obviously depends on three factors—the number at a birth, the frequency of reproduction, and the total number of successful gestations an animal may undergo. Unfortunately flock book records give available data on the first point only, although for specific cases some evidence on the second point (barring abortions and unregistered progeny) exists.

For breeding purposes the number of successful gestations is not a practical selective index, since the breeder can not afford to withhold progeny from breeding until their dams or sires shall have completed their breeding cycles. Frequency of reproduction or regularity of breeding as termed by the breeder is a more practicable trait for purposes of selection, but since barren reproductive periods are so much more frequently due to pathological or physiological causes than to genetic, most sheepmen lay principal emphasis on the number of offspring at the given birth.

There are two ways in which selection on this basis may be applied. The ewe may be selected on the basis of

a particular lambing, or the basis of the best lambing she shows. From a genetic standpoint the second criterion would seem the better, but practical breeders would be very likely to use the first. Unfortunately, records in Southdowns on which a comparison can be based are few, forty-three animals only being available. Table I presents the correlation of each individual record with the average lambing record for each ewe, while Table II presents the correlation of the best record for each ewe with her average. Nine of the ewes had four lambings to their credit, nine had three, while twenty-five had only two. The inadequacy of these data is recognized, since there is a false agreement between a single number and its average with another as compared to its agreement with its average with several numbers. Since, however, the material is suggestive from a comparative standpoint, it is presented, as the same actual error exists in each table:

TABLE I

CORRELATION OF INDIVIDUAL LAMBING WITH AVERAGE LAMBING PER EWE

Average Lambing per Ewe

Individual	1	1.5	1.67	1.75	2	2.5	F
1	38	6	4	4	1	0	53
2	0	6	8	12	31	1	58
3	0	0	0	0	1	1	2
Total	38	12	12	16	33	2	113

The coefficient of correlation for this table is 0.81806 ± 0.02099.

TABLE II

CORRELATION OF BEST LAMBING RECORD WITH AVERAGE OF EACH EWE

Average Lambing of Ewe

High Record	1.00	1.50	1.67	1.75	2.00	2.50	F
1	16	0	0	0	0	0	16
2	0	4	4	4	13	0	25
3	0	0	0	0	1	1	2
Total	16	4	4	4	14	1	43

The coefficient of correlation for Table II was found to be 0.92354 ± 0.01513.

While both records show a higher agreement with the average than probably exists in actual selections, the fact that the best record is more closely in agreement with the average than a random record makes high production a significant selection standard. The correlation between random records and the best records is presented in Table III.

TABLE III

BEST RECORD OF EWE

Individual Record	1	2	3	F
1	38	14	1	53
2	0	55	3	58
3	0	0	2	2
Total	38	69	6	113

The coefficient of correlation here is 0.6518 ± 0.03665. The relationship is not as great as between either of the records and the average record, as shown in Tables I and II. Since the correlation is not as high, and since an error (false agreement with the average) is introduced into each of the first two tables, it is well to determine whether the difference between the first two correlation coefficients is significant. Using the formula for the standard deviation of the difference between the first two constants presented by Pearl (10) : Error of $(x - y) = \sqrt{E_x^2 + E_y^2 - 2\, r_{xy}\, \sigma_x\, \sigma_y}$, where E refers to the error, x to the larger constant, y to the smaller, and r_{xy} to the correlation between x and y. The error of the difference between the correlation coefficients of Tables I and II is 0.01598. Since the difference is .10548, it is greater than three times the probable error, hence it is justifiable to conclude that the highest number at a birth is a better indication of the average fertility of an animal than a random birth, although on the basis of the figures presented the latter relationship is high.

Youatt (14) reports in 1837 that one ewe out of five in the average English flock produced twins, which would give 120 per cent. of lambs as the proportion of English flocks at that time.

Mansell (12) reports 168 per cent. of lambs in 11,668 English Shropshires in 1896, while Humphrey and Kleinheinz (6) from figures on the University of Wisconsin flock made the following breed comparisons:

TABLE IV

Breed	Singles		Twins		Triplets	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
Shropshire.....	42	23.7	120	67.8	15	9.5
Dorset.....	10	33.3	20	66.7	0	0.0
Southdown.....	27	38.0	44	62.0	0	0.0
Oxford.....	3	13.6	16	72.8	3	13.6
Hampshire.....	9	31.0	20	69.0	0	0.0
Cheviot.....	9	31.0	20	69.0	0	0.0

Percentage of lambs as given by Mansell is, of course, only a rough indication of twin-bearers, since ewes having triplet and quadruplet births may be included.

Rietz and Roberts (13) show that 43 out of every 100 births in American Shropshires are multiple births, while they have determined from Heape's (5) statistics of 1895–96 that 64 out of every 100 births in English Shropshires are multiple.

Plumb (12) found in 20,037 Shropshire births 59.2 per cent. were singles, 39.2 per cent. twins, and 1.3 per cent. triplets, all recorded in the American Shropshire Flock Book, 1890 to 1899.

Heape (5), from a study of the birth records of 89,000 ewes in English flocks, presents the following data to show the relative fertility of different breeds of sheep:

TABLE V

Breed	Per Cent. Lambs per Ewe	Per Cent. Twin Bearing Ewes
Suffolk	141.77	52.22
Kent	124.05	31.38
Southdown	109.89	18.67
Hampshire	114.69	24.09
Oxford Down	119.16	35.02
Dorset Horn	123.63	37.55
Shropshire	136.79	46.84
Lincoln	111.10	29.09

The figures for the per cent. of lambs per ewe and the per cent. of twin-bearing ewes do not in all cases check each other, as records of certain ewes were available for the one column but not for the other.

The writers tabulated birth frequencies in Shropshires, Cotswolds and Dorsets with the following results:

TABLE VI

Breed	Singles		Twins		Triplets		Quadruplets	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
Shropshire.....	10,585	69.41	4,561	29.91	102	.67	2	.01
Dorset.....	2,143	67.75	956	30.22	57	1.88	7	.22
Cotswold.....	5,523	79.24	1,431	20.53	16	.23		

Dorsets seem to have an exceptionally high percentage of triplets and quadruplets.

Factors Affecting Fertility

Heape (5) in a study of 122,673 breeding ewes, 413 English flocks, suggests five physiological factors that may affect the hereditary expression of fertility. The most important factor according to him is the physical condition of the ewe, which must be vigorous and healthy, especially at tupping (mating) time. The second most important factor is the feeding of the ewe, especially flushing previous to breeding, and careful diet during gestation. The third factor in importance is the district; he cited the fact that the Suffolk in its native country produced 60.46 per cent. of twins, while in Essex it produced only 42.87 per cent. The fourth factor in importance he found to be the age of the ewe; and the fifth, the season of year at which mating occurred.

Carlyle and McConnel (3) at Wisconsin discuss time of mating and age of ewe, factors similar to those mentioned by Heape. In a study of twelve years of records of the station flock at the University of Wisconsin they found that ewes bred early in the season dropped a higher percentage of lambs than those dropped late in the season,

while ewes from three to six years of age seemed to be at the optimum breeding period of their life. Humphrey and Kleinheinz (6) found that two-year-old ewes produced 141 per cent. of lambs and six-year-olds, 191 per cent. Possibly the writers do not understand the tables presented by them, but their calculations on the basis of the data there given would show the following averages at each age:

TABLE VII

Age	Average No. per Birth	No. Cases
2	1.54	53
3	1.56	52
4	1.71	52
5	1.73	57
6	1.92	24
7	1.45	11
8	1.00	2
9	2.00	2

Pearl (9) made a biometric study of the fertility of a long-lived ewe whose breeding record was as follows:

TABLE VIII

		Lambs		Lambs
April,	1806	1	1816	2
	1807	1	1817	2
	1808	2	1818	2
April 3,	1809	3	1819	2
Mar. 29,	1810	3	1820	2
	1811	3	1821	1
	1812	3	1822	1
	1813	3	1823	0
	1814	3	1824	0
	1815	2	Total	36

Assuming that the ewe was about one year old when the first lamb recorded was born, Pearl found that the mean point of the ewe's effective breeding life was 8.57 years, that the median point was 8.17 years and that the modal breeding point, or the point of maximum fertility per unit of time, was at 7.34 years.

Taking into account the seventeen years in which some young were born, the following constants regarding the number of lambs per birth were found:

Mean number of lambs per birth, 2.12 lambs.

Standard deviation in number of lambs per birth, 0.76.

Coefficient of variability in number of lambs per birth, 35.78 per cent.

Marshall (8) found after a study of the lambing statistics for various flocks of Scottish sheep for the years 1905, 1906, and 1907, that the percentage of lambs born was, as a general rule, highest among sheep which had been subjected to a process of artificial stimulation by means of special diet at the approach of the breeding season. In some cases the number of lambs per ewes in the "flushed" flocks was nearly 200 per cent. Flocks which were run upon special pasture upon the approach of the tupping season generally produced a slightly larger percentage of lambs than those receiving no sort of special feeding. Evvard (4) found among range ewes fed the same ration that the fourteen heaviest gaining ewes at time of breeding in his flock averaged 1.8 lambs; the fourteen medium gainers, 1.59; and the fourteen lightest gainers, 1.44.

Relation of Mammæ to Fertility

Alexander Graham Bell (2) conducted an experimental investigation on the relation of the number of mammæ to fertility. An unusually high fertility among a flock of native sheep in Beinn Breagh, Nova Scotia, led Bell to examine the ewes in order to discover some distinguishing mark of the twin-bearing ewe. He found a certain number of ewes with one to two supernumerary nipples in an embryonic, functionless condition. Of these abnormally nippled ewes, 43 per cent. had twin lambs, while of the normally nippled ewes but 24 per cent. produced twins. This apparent correlation between multinipples and increased fertility led to an extended series of experiments to ascertain whether by selective breeding, the supernumerary nipples could be made functional, and whether ewes with additional mammæ in a functional condition were more fertile than ewes with the normal number of nipples.

No difficulty was experienced in obtaining ewes that produced milk from six nipples. These multi-nippled sheep, however, did not prove to be more fertile than normally nippled sheep. In his 1912 paper (2) he states that the indications are that the six-nippled stock will ultimately prove to be twin bearers, as a rule, at maturity.

METHOD OF OBTAINING DATA

The source of the data in the present study was the American Southdown Record, the first twelve volumes being used to obtain cases of triplets, and volumes nine to twelve for twins and singles. The pedigree of each animal was reported into the third generation, recording the numbers of offspring at the birth of each animal. Some records on triplets were also taken from the American Shropshire Record, while Volume 25 was used to determine the ratio of singles, twins and triplets, Volumes 9–12 of the Southdown Flock Book for the same purpose, Volumes 12, 13, and 14 of the Continental Dorset Club Record, and Volumes 11 and 12 of the American Cotswold Record.

RELIABILITY OF FLOCK BOOK DATA

Records of the number at a birth in sheep are probably highly accurate for such material, since there is no observable tendency to discriminate in favor of, or against, recording offspring of multiple births, except the indirect one of lesser development in offspring from multiple births. This would not affect the reliability of the figures presented by the flockmaster, except perhaps to reduce slightly the proportion of multiple births registered. It may be safely assumed that the bulk of the records are accurate, barring clerical error.

THE NUMBER AT A BIRTH AS A GENETIC INDEX

Due to the physiological causes limiting the full expression of the genetic fertility of an animal it is obvious that animals recorded as singles may be potentially twin or

triplet bearers, or that ewes recorded as bearing twins may be genetically triplet producers or better. Hence it may be expected that not all single or twin bearers are alike in zygotic make-up with reference to fertility or that their breeding performance will fall into sufficiently well-defined categories to permit a rigorous Mendelian grouping. The relation between a random lambing record and the average record of ewes was shown earlier in this paper to be high, hence a similar relation might be expected to hold for true genetic fertility, were it measurable, and a random record.

The Data Involved

The Relative Influence of Sire and Dam.—Rietz and Roberts (12) found a mathematically significant effect of the sire on the number at birth as adjudged by the correlation between offspring and sires, although they do not find a similar relation between dams and maternal grandsires. While the authors have not secured correlation coefficients on this point, their averages may be so arranged as to throw some light on the same point. Using pedigrees which were started from animals of single birth, the following comparison between sires and dams is possible.

TABLE IX

Relative Influence of Sire and Dam on Birth Number, from Pedigrees of Animals of Single Birth

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
1,872	1	1	1.29	1,872	1	1	1.29
925	1	2	1.28	570	2	1	1.25
14	1	3	1.43	12	3	1	1.50
5,570	2	1	1.25	925	1	2	1.28
306	2	2	1.34	306	2	2	1.34
10	2	3	1.20	6	3	2	1.17
12	3	1	1.15	14	1	3	1.43
6	3	2	1.17	10	2	3	1.20

Comparison of the records of single, twin and triplet sires mated to single, twin and triplet ewes in the preceding table shows no particular influence of the birth rank of the sire, a fact which is confirmed in Table X, where the average performance of each is given.

TABLE X

BREEDING PERFORMANCE OF THE MALES FROM PEDIGREES STARTED WITH SINGLE BIRTHS

Sires	Mean	Standard Deviation	No. Cases
1.....	1.2864±.00593	.4668	2,811
2.....	1.2776±.01031	.4553	886
3.....	1.3888±.07750	.4875	18

The difference between the breeding performance of the singles and twins is 0.0088 ± 0.0119, which is, of course, not sufficient to be significant. It indicates either that the male has no influence on the number at a birth (the most probable supposition) or that singles and twins in the males are genetically similar. The difference between the breeding performance of the triplets and singles is $0.1024 \pm .0777$ and between the triplets and twins is $0.1112 \pm .07818$, neither of which is significant.

For the ewes the result is not particularly different. Table XI presents the result of this comparison.

TABLE XI

BREEDING PERFORMANCE OF THE FEMALES FROM PEDIGREES STARTED WITH SINGLE BIRTHS

Dams	Mean	Standard Deviation	No. Cases
1.....	1.26365±0.00631	.4635	2,454
2.....	1.29345±0.00893	.4658	1,237
3.....	1.33333±0.06490	.4714	24

The difference between the progeny of ewes born singly and those born twins is $.01349 \pm 0.1093$; between singles and triplets is $.05338 \pm 0.06252$; and between twins and triplets is $.03989 \pm 0.0655$.

TABLE XII

RELATIVE INFLUENCE OF SIRE AND DAM ON BIRTH NUMBER, FROM PEDIGREES OF ANIMALS OF TWIN BIRTH

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
2,805	1	1	1.51	2,805	1	1	1.51
1,294	1	2	1.55	687	2	1	1.57
21	1	3	1.86	19	3	1	1.68
687	2	1	1.57	1,294	1	2	1.55
468	2	2	1.56	468	2	2	1.56
10	2	3	1.60	7	3	2	1.43
19	3	1	1.68	21	1	3	1.86
7	3	2	1.43	10	2	3	1.60

Table XII shows the relative breeding performance of the sires and dams in pedigrees started from twin births.

Treating the sires in pedigrees from twin births as in Table X, Table XIII is produced.

TABLE XIII

BREEDING PERFORMANCE OF THE MALES FROM PEDIGREES STARTED WITH TWIN BIRTHS

Sires	Mean	Standard Deviation	No. Cases
1.....	1.5296 ± .00543	.51659	4,120
2.....	1.5682 ± .00724	.49704	1,165
3.....	1.6154 ± .06435	.48650	26

The difference between singles and twins as sires is .0386 ± .0091; between singles and triplets is .0858 ± .0645; and between twins and triplets is .0472 ± .0647.

The difference between singles and twins is in this case significant, being about 4.2 times the probable error. Further consideration will be given this difference when the ewes are discussed.

Treating the ewes in pedigrees from twin births as in Table XI, Table XIV is produced.

TABLE XIV

BREEDING PERFORMANCE OF FEMALES FROM PEDIGREES STARTED WITH TWIN BIRTHS

Dams	Mean	Standard Deviation	No. Cases
1.....	1.2529±.00581	0.51075	3,511
2.....	1.5551±.00827	0.51583	1,769
3.....	1.7742±.05065	0.41811	31

The difference between singles and triplets is 0.2513 ± .05098; between twins and triplets, 0.2191 ± .05132; and between singles and twins is 0.0322 ± .01011. Ewes from triplet births give significantly larger progenies than ewes from single or twin births, while ewes from twin births give significantly larger progenies than ewes from single births, the last difference being 3.323 times the probable error. It is interesting to observe that both

twin rams and twin ewes are significantly better breeders than singles. Just why this result is obtained here in the face of other contradictory data is difficult to understand.

In order to combine the results of the two types of pedigrees it was deemed advisable to utilize the ratio of 1:4.118 twins to singles discovered by examination of volumes 9 to 12, respectively, in order to have the normal relationship between twins and singles. This involved dividing the numbers of individuals in the twin group or multiplying those in the single group. In the first case errors would be increased, due to the elimination of certain groups, while in the second case errors would be increased due to the exaggeration of differences between the random sample in the pedigrees begun from single births and the normal distribution of such a population. It was deemed best to use the second method, since it permitted the retention of the small groups, hence the ratio 1:4.118 was multiplied by the ratio 3,715:5,311, the numbers of individuals in the pedigrees from twin and single births, respectively, which gave the multiplying factor 5.887 for the pedigrees started from single births. Of course, this result is only suggestive; but it was impractical to record the additional 4,300 odd pedigrees necessary to get a true random distribution. Treated this way, multiplying Table V by 5.887 and adding to Table IX, Table XV is produced.

TABLE XV

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
13,826	1	1	1.33	13,826	1	1	1.33
6,739	1	2	1.33	4,043	2	1	1.33
1,103	1	3	1.51	89	3	1	1.54
4,043	2	1	1.30	6,739	1	2	1.33
2,270	2	2	1.38	2,270	2	2	1.38
69	2	3	1.26	42	3	2	1.21
89	3	1	1.54	1,103	1	3	1.51
43	3	2	1.21	69	2	3	1.26

Treating the sires as in Tables VI and X, Table XVI is produced.

TABLE XVI

BREEDING PERFORMANCE OF THE MALES GIVEN IN TABLE XI

Sires	Mean	Standard Deviation	No. Cases
1.....	1.3340±.00229	.48658	20,668
2.....	1.3308±.00403	.47677	6,382
3.....	1.3318±.2908	.49533	132

The difference between singles and twins is .0032 ± .0463; between singles and triplets, .0978 ± .02916; and between twins and triplets, .1010 ± .02936.

TABLE XVII

BREEDING PERFORMANCE OF FEMALES FROM TABLE XV

Dams	Mean	Standard Deviation	No. Cases
1.....	1.3274 ± 0.00243	0.48261	17,958
2.....	1.3444 ± 0.00345	0.48713	9,052
3.....	1.4128 ± 0.02533	0.49234	172

The difference between singles and twins is found to be 0.0170 ± 0.00422; between singles and triplets, 0.0854 ± 0.02545; and between twins and triplets, 0.0684 ± 0.02556. Several of the differences in Tables XV and XVI verge on significance, being at least three times the probable error.

RELATIVE INFLUENCE OF MALE AND FEMALE IN GRANDPARENTS

From the study of the relative influence of the sires and dams on the progeny it would seem fruitless from biometric grounds to look for transmission through one sex more than the other. Yet logically it would seem that the grandsire and grandam on the dam's side would have a more potent effect on the birth number from the dam than would the paternal grandparents. Studies of this sort are available from the pedigrees. Perhaps the first concern is to determine the relation of the birth rank of the grandparents to that of the progeny. Table XVIII presents this information.

TABLE XVIII

RELATION OF BIRTH FREQUENCIES IN GRANDPARENTS TO BIRTH FREQUENCIES OF PROGENY

Grandsire	Grandam	Ave. Birth Ranks	Ave. Progeny	Standard Deviation	No. Cases
1	1	1	1.6500±.00881	.5451	1740
1	2	1.5	1.7041±.01488	.6030	747
2	1	1.5	1.7065±.01923	.5717	402
2	2	2	1.7500±.03328	.5517	308
1	3	2	1.8095±.08642	.5871	21
3	1	2	2.0000±.07855	.0000	6
2	3	2.5	1.6667±.08297	.5634	21
3	2	2.5	1.7500±.14606	.4331	4

The difference between the average progeny from grandparents 1♂ × 1♀ and grandparents 2♂ × 1♀ is .0579 ± .02114. This is not three times the probable error, therefore the difference is not significant. The difference between grandparents 1♂ × 1♀ and grandparents 1♂ × 3♀ is .1609 ± .08687. This also is less than three times the probable error, hence is insignificant. In fact none of the differences are significant.

To determine whether birth rank in males or females among the maternal grandparents has effect on transmission, they were compared in the same manner as the sires and dams were. The results for grandsires are:

TABLE XIX

RELATION OF BIRTH RANK OF GRANDSIRE TO BIRTH RANK OF PROGENY

Birth Rank of Grandsire	Ave. Progeny	Standard Deviation	No. Cases
1	1.6675±.00760	.5643	2,508
2	1.7209±.01442	.5779	731
3	1.9000±.06399	.3000	10

The difference between singles and twins is .0534 ± .0163; between twins and triplets is .1791 ± .06559; and between singles and triplets is .2125 ± .06444. The difference between singles and triplets is 3.62 times the probable error, while the difference between singles and twins is 3.34 times its probable error.

Treating the dams in the same manner as the sires Table XX is produced:

TABLE XX

RELATION OF BIRTH RANK OF GRANDAM TO BIRTH RANK OF PROGENY

Birth Rank of Grandam	Average Progeny	Standard Deviation	No. Cases
1.....	1.6615±.00737	.5064	2,148
2.....	1.7177±.01239	.5979	1,059
3.....	1.7381±.06034	.5798	42

The difference between singles and twins is .0562 ± .01435; between singles and triplets is .0766 ± .06079; and between twins and triplets is .0204 ± .06159. The only significant difference is between singles and twins, which is 3.88 times the probable error.

The probable errors involved seem to indicate little, hence a comparison by correlation of the maternal grandsire and progeny, and maternal grandam and progeny was instituted. Table XXI presents the correlation for the maternal grandsire, Table XXII for the maternal grandam.

TABLE XXI

CORRELATION OF MATERNAL GRANDSIRE AND PROGENY

Birth Rank Grandsire	Birth Rank Progeny				*f*
	1	2	3	4	
1.....	954	1,435	118	1	2,508
2.....	251	431	49		731
3.....		9	1		10
f.....	1,205	1,875	168	1	3,249

TABLE XXII

CORRELATION OF MATERNAL GRANDAM AND PROGENY

Birth Rank Grandsire	Birth Rank Progeny				*f*
	1	2	3	4	
1.....	811	1,254	82	1	2,148
2.....	381	596	82		1,059
3.....	3	25	14		42
f.....	1,195	1,875	178	1	3,249

The coefficient of correlation for Table XXI is .0496 ± .0118, while for Table XXII it is .0382 ± .0118. The difference between the correlations of maternal grandsire

and grandam is .0114 ± .0167, a difference insignificant, hence one can not assume sex linkage.

EXAMINATION OF SHROPSHIRE DATA FROM THE MENDELIAN STANDPOINT

A number of Shropshire pedigrees were tabulated which were all started from triplet births. It had seemed from inspection that triplets might be genetically different from twins and singles, hence the pedigrees were tabulated to discover such a difference if possible. If the maternal grandparents affected the number at a birth from their daughter, then it was possible that certain differences might appear in the pedigrees indicating the genetic effects. The results follow.

TABLE XXIII

RELATION OF BIRTH RANK IN OFFSPRING TO BIRTH RANK IN DAM WHEN THE MATERNAL GRANDPARENTS ARE SINGLES

Offspring

Dam	1	2	3	4	Mean	Standard Deviation
1.....	17	16	17	0	2.00	0.824
2.....	12	10	15	1	2.13	0.894
3.....	0	0	1	0	3.00	0.000

TABLE XXIV

WHEN MATERNAL GRANDSIRE IS A SINGLE AND MATERNAL GRANDAM IS A TWIN

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	15	9	15	2.00	0.873
2.....	10	9	25	2.34	0.825
3.....	5	0	0	1.00	0.000

TABLE XXV

MATERNAL GRANDSIRE A TWIN, MATERNAL GRANDAM A SINGLE

Dam	1	2	3	Mean	Standard Deviation
1.....	8	3	13	2.21	0.912
2.....	7	5	7	2.00	0.858
3.....	1	0	0	1.00	0.000

TABLE XXVI

Maternal Grandparents Twins

Dam	1	2	3	Mean	Standard Deviation
1.....	4	2	4	2.00	0.895
2.....	9	9	9	2.00	0.817
3.....	0	1	2	2.67	0.417

TABLE XXVII

Maternal Grandsire a Triplet, Maternal Grandam a Single

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	0	1	0	2.00	0.000
3.....	0	0	0	0.00	0.000

TABLE XXVIII

Maternal Grandsire a Single, Maternal Grandam a Triplet

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	0	0	1	3.00	0.000
3.....	0	0	1	3.00	0.000

TABLE XXIX

Maternal Grandsire a Triplet, Maternal Grandam a Twin

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	1	0	0	1.00	0.000
3.....	0	0	0	0.00	0.000

TABLE XXX

Maternal Grandsire a Twin, Maternal Grandam a Triplet

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	1	3.00	0.000
2.....	0	1	1	2.50	0.500
3.....	0	0	0	0.00	0.000

Since all the pedigrees were started from triplets the excess of triplets is so great as unduly to weight the ratios. Inspection of the ratios does not reveal any particular difference in the progeny descended from a particular pair of grandparents, whether the dam is a single, twin or triplet. Since also there seems to be no sex linkage involved it seemed desirable to combine similar matings from the standpoint of birth rank. The totals produced are presented in Table XXXI.

TABLE XXXI

SUMMARY OF TABLES XXIII TO XXX WITH RESPECT TO BIRTH RANK OF MATERNAL GRANDPARENTS

No. Offspring

Birth Rank Maternal Grandparents	1	2	3	4	Mean	Standard Deviation
Both grandparents single...	29	26	33	1	2.07	0.85851
One grandparent a twin....	46	26	60	0	2.11	0.88983
Both grandparents twins...	13	12	15	0	2.05	0.83516
One grandparent a triplet..	1	2	4	0	2.43	0.70855

Confirmation of the previous view that twins and singles are genetically alike, while triplets differ from either, seems to be found in Table XXXI. However, the difference between triplets and the mating where one grandparent is a twin is only 0.32 ± 0.20. This is not three times the probable error, but by consulting Pearl and Miner's (11) table it is found that the chances that the difference is significant are about two and a half to one.

CONCLUSIONS

1. In general sheep of a high birth rank tend to produce offspring of a high birth rank.
2. On the basis of the few data presented, the highest record of a ewe appears to be a better selection standard for high fertility than a random record.
3. The frequency of multiple births in sheep varies with the breed.
4. Physiological factors may exert a marked influence

on heredity, the most important factors being the vigor of ewe, the feeding of ewe, the age of ewe, the season and the region.

5. Apparently no relation exists between high fertility and additional mammæ.

6. In pedigrees started from single births, the birth rank of the sire does not affect the birth rank of the progeny; in pedigrees started from twin births, the effect of high birth rank of the sire is only slightly significant (more than three times the probable error).

7. The effect of birth rank of ewe on the birth rank of progeny is the same as that of the sire except in the case of pedigrees started from twin births when it is slightly greater.

8. No evidence for a sex linkage of fecundity factors occurs in the pedigrees tabulated, as shown by a comparison of the relative influence of progeny of the maternal grandam and the maternal grandsire.

9. Evidence from Shropshire triplet pedigrees suggests that triplets are genetically different from twins and singles, which two are probably genetically alike.

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LINKED QUANTITATIVE CHARACTERS IN WHEAT CROSSES

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SINCE wheat has but 8 chromosomes in the sexual cells and since the parents in the macaroni bread wheat crosses here discussed certainly differ in more than 8 visible characters, it was thought likely that a genetic linkage of some of these might be found. The following study is an endeavor to discover whether or not there is such a linkage between the shape of the head, *i. e.*, ratio of width of head (measured parallel to the face of the head which shows the sinuous furrow formed between the two rows of spikelets) to the thickness (measured parallel to the array of seeds in the spikelet) and the texture (translucency or opaqueness) of the grain. Of the two parents here discussed No. 1, the macaroni wheat, had a much flattened head and very hard translucent grains, whereas the other parent, No. 35 (a bread wheat), had a nearly square head with soft opaque grain.

The seed on the F_1 plants of this cross were all wrinkled and intermediate in texture between the two parents, *i. e.*, they were dull, being neither translucent like the macaroni parent nor opaque like those of the bread wheat parent. In order to make a quantitative expression of hardness the perfectly translucent grains of the macaroni parent were called 100 per cent. hard, the seeds of the F_1 50 per cent. hard and those of the bread wheat parent were called 0 per cent. hard. Since in the seeds of the F_2 and F_3 plants every possible degree of intergradation occurred between the characters of the two parents and since any form of classification adopted would be purely arbitrary, it was decided to make it the most simple possible and place all of the variants into three groups by the following means: a grain that was approximately as hard

TABLE I. CORRELATION BETWEEN {WIDTH / THICKNESS} OF HEAD AND TEXTURE OF GRAINS. 1914

Ratio	{Width/Thickness} of Head															Average W./T.	Total No. Plants	Character of Grain
	.60 .69	.70 .79	.80 .89	.90 .99	1.00 1.09	1.10 1.19	1.20 1.29	1.30 1.39	1.40 1.49	1.50 1.59	1.60 1.69	1.70 1.79	1.80 1.89	1.90 1.99	2.00 2.09			
Pure No. 1							3	7	12	46	*34*	23	21		4	1.63	150	Hard
1 × 35 F_1						1	1	5	4	*9*	12	3	4			1.56	39	Intermediate
Pure No. 35	1	4	*3*	1	2	1										.88	12	Soft

TABLE II. CORRELATION BETWEEN {WIDTH / THICKNESS} OF HEAD AND TEXTURE OF GRAINS. 1915

	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	250 259	260 269	270 279	Average W./T.	Total No. Plants	Character of Grain
Pure No. 1				1		5	8	17	22	36	*55*	55	56	32	3	4	2	1						1.57	298	Hard
Pure No. 1				1	1	23	26	26	39	37	*58*	58	47	21	5	6	1	1		1				1.55	352	Intermediate
1 × 35 F_2			2	8	8	29	40	78	85	55	*100*	88	64	67	3	28	25	7	2	2	1		1	1.55	693	Hard
1 × 35 F_2	2	3	7	28	44	161	196	*204*	175	96	120	68	48	47	1	17	3	4	1	1				1.29	1,226	Intermediate
1 × 35 F_2		5	10	34	61	96	*52*	36	15	11	7	3	1	1										1.11	332	Soft
Pure No. 35		3	14	40	*55*	41	13																	.95	166	Soft

TABLE III. CORRELATION BETWEEN {WIDTH / THICKNESS} OF HEAD AND TEXTURE OF GRAINS. 1916

	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	250 259	260 269	270 279	Average W./T.	Total No. Plants	Character of Grain
Pure No. 1				3	1	14	17	20	36	*40*	65	34	20	2	1									1.45	253	Hard
						1	3	1	8	10	*20*	9	2	1										1.50	55	Intermediate
1 × 35 F_2			2	4	5	44	88	153	184	225	*475*	322	291	215	40	140	55	23	4	2	3			1.59	2,275	Hard
1 × 35 F_2			3	9	14	120	198	313	*206*	116	171	53	38	20	1	20	4	5						1.33	1,291	Intermediate
1 × 35 F_2			6	7	19	147	208	*338*	177	66	64	16	8	2		5								1.24	963	Soft
Pure No. 35	1	5	12	25	38	*71*	63	25			1													1.04	241	Soft

as the macaroni parent was called 100 per cent. hard; a grain as soft as the bread wheat parent was called 0 per cent. hard; all grains intermediate between these were called 50 per cent. hard. A plant which produced 60 per cent. hard grains, 30 per cent. intermediate grains and 10 per cent. soft grains would therefore be classified as follows:

$.60 \times 1.00 + .30 \times .50 + .10 \times 0 = .75 = 75$ per cent. hard.

Spotted grains (grains containing well-defined areas of opaque starch in an otherwise translucent grain) such as occur frequently in the macaroni wheat and also among the hybrids were treated as intermediate grains. After classifying the seeds of each plant in this manner all those which were over $66\frac{2}{3}$ per cent. hard were termed hard wheats, those from $33\frac{1}{3}$ to $66\frac{2}{3}$ per cent. hard were classed as intermediate and all less than $33\frac{1}{3}$ per cent. hard were classed as soft.

In 1914 the two parents (Nos. 1 and 35) and F_1 of this cross were grown with the following results:

The ratio, W./T. of the F_1 plants of this cross, is thus seen to be much nearer to the macaroni parent both in average and distribution than it is to the bread wheat parent.

Table II shows this same study made for the F_2 plants, grown in 1915.

A uniform correlation between the hardness of the grain and the ratio of width to thickness of head is apparent in Table II.

Table III gives the results of this same study for the crop of 1916. There is again apparent a marked correlation between the ratio of width to thickness of head and the texture of the grain. It will be noticed that both in 1915 and 1916 the soft (0 per cent. hard) hybrids had a higher average ratio W./T. than the sonora. It should, however, be remarked that the hybrid softs were also harder on the average than the pure number 35, but since

TABLE IV

DISTRIBUTION OF PLANTS WITH REGARD TO THEIR PERCENTAGE OF HARD GRAINS AND THE RATIO OF WIDTH TO THICKNESS OF HEAD

	Pure No. 1 in 1915											Pure No. 1 in 1916										
	Percentage of Hard Grains											Percentage of Hard Grains										
	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46
244 240	1																					
239 235																						
234 230																						
229 225								1														
224 220						1																
219 215																						
214 210			1		1				1													
209 205																						
204 200	1	1		1	1			2	1	1	2											
199 195																						
194 190	1		1		1			2	2	1			1									
189 185	5		1	2	2	3	1	3	6	2	1											
184 180	1	2	4	3	1	4	3	2	4	3		2								1		
179 175	3	2	4	1	7	5	4	6	9	8	1											
174 170	3	2	5	3	3	8	6	10	10	4	1	10	3	3	2	1	1			2		
169 165	3	2	2	3	1	1	1	4	2	12	4	4		2		1		1				
164 160	6	2	7	4	6	9	8	9	11	10		11	1	5	3	5	2	5	1	1	1	
159 155	6	4	2	4	3	4	8	10	1	9	3	8	2	2			1		1			
154 150	3	3	5	4	3	2	4	6	11	14	4	25	4	7	5	5	6	4	7	4	4	
149 145	2	5	2	1	4	5	6	3	4	10	3	6	2	3	1	1			1			
144 140	1	1		1	2	4	2	3	2	4	8	11	9	5		1	1	3	4		1	1

	Pure No. 1 in 1915											Pure No. 1 in 1916										
	Percentage of Hard Grains											Percentage of Hard Grains										
	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46
139																						
135	3	2		2	1	2	2	4	5	7	7	13	4	1	1	2			2	2	1	
134																						
130	1	1	1	3		2	2	3	3	8	2	5	4	1	2	1	2	2	1			
129																						
125		2	1	3		2	3	1	4	11	8	11	1	3	1		1					
124																						
120	1		1		1		1			1	1	5	1	1					1			
119																						
115		1	2	2	1	1	1	2	3	16	5	12	3		1				1			1
114																						
110												1						1				
109																						
105				1		2		1	2	9	5	4		1								
104																						
100				1	1					2	4	5	3			1		1				
99																						
95																						
94																						
90											1	1										
89																						
85												1										
84																						
80		1								1					2							

they were opaque and graded insensibly into the condition of pure No. 35 it was considered impracticable to make the arbitrary designation of degrees of hardness any more complex than the three groups used. It should be added, on the other hand, that a number of races were secured which were as soft or softer than the type of No. 35 and a number of races which, to all appearances, were fully as hard as pure No. 1. The inheritance of hardness will, however, be reserved for discussion in a later paper.

The question now arises as to whether this correlation is genetic or physiological. Might it not be caused by the simple fact that poorly filled (with starch) hard grains will give rise to a more flattened head, than will plump (starchy, soft) grains by failing to fill up and distend the

glumes? This question can be answered by studying the correlation between the flattening of head and hardness of grain in a pure race. Since all of the No. 35 was soft, this study could not be made for the soft wheat, but as there were many plants of the pure macaroni which produced a greater or less proportion of soft grains, a comparison was possible. That the plants producing a large proportion of hard grains on an average did not have more flattened heads than those which produced a greater proportion of soft grains is shown in Tables II and III by comparing the "hard" with the "intermediate" groups of pure No. 1. This is perhaps better shown in Table IV, where the distribution of the plants is made with regard to their hardness per cent. and the ratio of width to thickness of head.

It should be noted from Tables II and III that there are numbers of individual plants with low ratios of width to thickness of head, but with high percentages of hard grains. It is difficult to see how this could occur if the low ratio was due simply to the lack of plumpness of the hard grain. *Moreover, races of hard macaroni wheats occur which have approximately square heads, and there are varieties of soft wheats* (*Little Club*) *with rather strongly flattened heads.* In 1916 there were a few cases where hybrid races having low average ratios (W./T.) also were rather high in average per cent. of hard grains. All of the races in 1916 having ratios averaging as low or lower than 1.35 and average percentages of 60 or more per cent. of hard grains are given in the following table:

It is thus seen that all of the races which on the average violated the correlation in 1916 came from plants which more or less markedly violated this same correlation in 1915. The cases given would indeed be hard to explain on a basis of violation of physiological correlations but if we are dealing with a genetic correlation, they may be easily explained on the "cross over" theory as used by Morgan for reversal of linkages in the characters of *Drosophila.*

TABLE V

INHERITANCE OF NON-CONFORMITY TO USUAL CORRELATION BETWEEN HIGH RATIO W./T. OF HEAD AND AVERAGE PERCENTAGE OF HARD GRAINS

No.	Parents in 1915		Offspring in 1916	
	W./T.	Per Cent H.	W./T.	Per Cent H.
132	.93	98	1.34	88
169	1.10	50	1.24	73
219	1.20	50	1.35	93
232	1.25	100	1.33	86
239	1.33	60	1.30	88
263	1.00	64	1.17	61
279	1.27	50	1.35	79

In the opposite direction, *i. e.*, races which markedly violated the correlation by having very high ratios (1.50 or more) but with low percentages of hard grains did not occur.

Again it may be objected that an explanation of this correlation between shape of head and texture of grain as a genetic linkage, is incorrect because the linkage is not complete, *i. e.*, there is considerable regression. This objection may be fully met by the observation that both of the characters here concerned are quantitative and hence subject to fluctuation around a mean. Moreover, it is almost certain that both characters are genetically compound, *i. e.*, each are the result of more than one factor, recombinations of which may markedly vary the quantitative visible expression of the characters. If, therefore, but a single factor for grain texture be linked with one of the factors concerned in the shaping of the head, there will result a partial correlation of these two characters such as we find.

The data here presented, therefore, seems to indicate that the two characters, hardness of grain and high ratio of width to thickness of head, which entered this cross together in the macaroni parent, tend to come out together in the segregates of the F_2 and F_3 generations, *i. e.*, that there is a genetic linkage between one or more of the factors controlling the grain texture and head shape in the two varieties employed as parents.

ON REVERSIBLE TRANSFORMABILITY OF ALLELOMORPHS

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IN genetical studies of variegation in plants, the fact has been observed occasionally that with a certain frequency a dominant allelomorph occurs in the corresponding recessive homozygote (De Vries,[1] Correns,[2] and Emerson[3]). In this paper the author presents a new instance of a similar phenomenon, which it is hoped may throw additional light on the subject.

In certain pedigree cultures of the rice plant, *Oryza sativa* L., there happened to occur in 1912 families containing besides ordinary fertile plants a number of sterile plants. These sterile plants were normal in their growth, but showed a considerable barrenness at the ripening season. Some of them yielded no seed whatsoever, others bore a small number of normal seeds, and very few were mosaic forms with higher fertility. These families, two in number, each belonging to a different variety, were derived from single plants of the former generation, and were very uniform in other characters. From them the experiment was started.

The rice plant, being a self-pollinated species, is convenient material for breeding experiments. Although the experiments in this investigation were made largely from open-pollinations, the results obtained were always similar to those from experiments in which plants were artificially protected against accidental natural crossing.

The observations of 1912 and 1913 are shown in summarized form in Tables I and II, *a* and *b*, and point to the following conclusions. Sterility behaves as a simple re-

[1] De Vries, H., "Die Mutationstheorie," Bd. I, 1901, pp. 489–511; "Species and Varieties, their Origin by Mutation," 1905, pp. 309–339.

[2] Correns, C., *Berichte der Deutschen Botanischen Gesellschaft*, Bd. 28, 1910, pp. 418–434.

[3] Emerson, R. A., AMERICAN NATURALIST, Vol. 48, 1914, pp. 87–115; *Genetics*, Vol. 2, 1917, pp. 1–35.

cessive to fertility, and the seeds resulting from partial fertility of sterile plants again give segregating families. In Family *A*, which shows an exceedingly slight fertility of sterile plants, the segregation ratio in the offspring derived from fertile individuals is quite close to expectation, but in Family *B* which shows a considerably higher grade of partial fertility of sterile plants, the progeny of fertile individuals exhibit considerable deviations from the expected segregation ratio.

TABLE I

THE SEGREGATING FAMILIES, *A* AND *B*, IN 1912

Fam.	Segregation						Partial Fertility of Sterile Plants		
	Fertile Plants	Sterile Plants	Totnl No. of Ind.	Steriles %	Ratio per 4		Total No. of Spikelets	Fertile Spikelets	
					D	R		No.	%
							ca.		
A	36	13	49	26.53	2.94	1.06	9,000	2	0.02
B	105	25	130	19.23	3.23	0.77	14,941	434	2.90

TABLE II

THE FAMILIES DERIVED FROM FAMILIES *A* AND *B*

(a) The Progeny of the Fertile Plants

Family in 1912	No. of Families			Ratio per 3		Segregating Families			
	Uniformly Fertile	Segregating	Total	Uniformly Fertile Families	Segregating Families	Fertile Plants	Sterile Plants	Total Number Individuals	Steriles %
A	10	22	32	0.94	2.06	1,068	346	1,414	24.46
B	41	64	105	1.17	1.83	4,874	1,301	6,175	21.06

(b) The Progeny of the Seeds on the Sterile Plants

Family in 1912	Number of Families	Fertile Plants	Sterile Plants	Total Number of Individuals	Steriles %
A	2	2	0	2	0.00
B	24	401	115	516	22.29

These facts may be interpreted by the following hypothesis. The dominant and the recessive types concerned are assumed to be transformed by certain unknown causes into the other allelomorph. The recessive allelomorph which has made its appearance in Families *A* and *B* is assumed to have originated in the preceding

generation by the transformation of the dominant allelomorph. This recessive state of the hereditary substance, however, has a tendency to revert into the original dominant state. Such reversion is especially likely to occur in vegetative cells, where each recessive allelomorph seems to be able to revert independently. Consequently, in recessive homozygotes the reversion generally will produce heterozygotic cells, either one of the two recessive allelomorphs being changed into the dominant. The heterozygotic cells thus formed will give rise to partial fertility in otherwise sterile plants. Again, the recessive allelomorph in heterozygotic cells may be subject to similar reversion, and such reversion may occur both in the heterozygotic cells of sterile plants and in normal heterozygotes. Here, however, heterozygotic cells will be transformed into dominant homozygotic cells without visible effect on the plant concerned. The consequence of this reversion in the next generation will be that the proportion of the dominant segregates may exceed the theoretically expected figure. Finally, it may be assumed that between Families *A* and *B* there exists a difference in the reverting tendency of the recessive allelomorph, which necessarily will effect the differences in both the intensity of partial fertility of sterile plants and the deviations in the segregation ratio.

In Table III the segregating families derived from the fertile plants of Family *B* are classified according to the magnitudes of the deviations in terms of probable errors. The true percentage for the recessive is assumed, in the one case as 25 per cent. (the Mendelian ratio), and in the other case as 21 per cent. (an arbitrary number). In comparing the two different frequency distributions made in this manner with the theoretical frequency distribution, it is observed that while the frequency distribution of the deviations from 25 per cent. shows a considerable discrepancy from the theoretical, the latter fits the frequency distribution of the deviations from 21 per cent. rather closely, the goodness of fit being $P = 0.915$. Consequently, the *ca.* 4 per cent. deficiency of recessive segre-

gates is a normal expectation and not an experimental error.

TABLE III

THE FREQUENCY DISTRIBUTION OF THE DEVIATIONS IN THE SEGREGATION RATIOS IN THE GROUP OF 64 SEGREGATING FAMILIES DESCENDED FROM FAMILY *B* OF THE YEAR 1912

Dev. /P.E.	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	Total
Experimental frequency (I)	1	7	14	17	15	6	4					64
Experimental frequency (II) ...		1	2	11	19	14	10	5	2			64
Expectation	0.2	1.2	4.3	10.3	16.0	16.0	10.3	4.3	1.2	0.2		64.0

Note: In the experimental frequency (I) the true percentage for recessives is taken as 25 per cent., and in (II) as 21 per cent.

Such an aberrant segregation ratio seems to be a constant tendency all through the generations descended from Family *B*. This is shown in Table IV in which the experiments in the years from 1912 to 1915 are summarized.

TABLE IV

THE ABERRANT SEGREGATION-RATIOS OBTAINED IN THE YEARS 1912–1915

Years	No. of Fams.	Parent-plants	No. of Inds.	Fertiles	Steriles	Ster. %	Dev. %	P.E. %	D./P.E.
1912	1	Fertile	130	105	25	19.23	5.77	2.55	2.3
1913	64	"	6,175	4,874	1,301	21.06	3.94	0.37	10.6
1914	10	"	1,560	1,207	353	22.63	2.43	0.74	3.3
1915	53	"	4,696	3,732	964	20.52	4.48	0.47	9.5
Total....	128		12,561	9,918	2,643	21.04	3.96	0.26	15.2
1913	24	Sterile	516	401	115	22.29	2.71	1.21	2.2
1914	34	"	994	779	215	21.63	3.37	0.93	3.6
1915	19	"	684	522	162	23.68	1.32	1.12	1.2
Total....	77		2,194	1,702	492	22.43	2.57	0.62	4.1

Again, in regard to the intensity of partial fertility of sterile plants, the descendants of Families *A* and *B* exhibited respectively relations similar to those seen in 1912. (Family *A* was not traced after 1913.) A count of fertile spikelets on sterile plants descending from Family *B* was made in 1914 on 281 plants bearing a total of 101,412 spikelets. In this count the number of fertile spikelets was 3,857, corresponding to 3.78 per cent. of the total number of spikelets. The latter figure may be regarded as the average fertility of sterile plants in the progeny of Family *B*.

The fertile spikelets of sterile plants are generally scattered at random over the panicle, and each fertile spikelet may be regarded as representing a separate case of reversion; but in mosaic forms which show higher fertility and are of rarer occurrence, the reversion may have taken place in earlier stages of plant development, resulting in larger fertile sections. Consequently, when the count of fertile spikelets is made with only the first type of sterile plants, a more correct value for the frequency of reversion may be obtained. The result of such a count on 902 panicles containing 93,635 spikelets is 1,858 fertile spikelets, *i. e.*, 1.98 per cent. of the total number of spikelets.

The mosaic forms appear in several different grades of partial fertility. In a panicle either one or more branches or even one half of the panicle can be highly or entirely fertile, the remaining part being absolutely or nearly absolutely sterile. Similarly, in a single plant some whole panicles can be entirely or highly fertile while others are of the ordinary grade of partial fertility. Furthermore, similar mosaic conditions were also observed in single flowers of sterile spikelets. While all six anthers of a sterile spikelet generally bear none or but few pollen grains, occasionally flowers appear in which certain anthers contain a considerable number of pollen grains of normal appearance and others show the ordinary state of sterility. Hence it may be assumed that the reversion can take place at any stage of plant development.

The partial homozygosity of heterozygotes, corresponding to the partial fertility of sterile plants, may be estimated in the following way. Assuming that the possibility of reversion at any stage of a plant's life, similar to that observed above, may also occur in heterozygotic cells, then we may distinguish for convenience two different types of reversions; there is the reversion which will cause partial homozygosity within a single flower, and the reversion which will produce an entirely homozygotic spikelet or larger homozygotic sectant. Suppose then that the latter reversion will give to the heterozygote

homozygotic (AA) spikelets in any part "x" of the total number of spikelets which is taken as a unit, and again that in the remaining (1 — x) part of the total number of spikelets, the other type of reversion will occur, turning some part "y" of the whole generative tissue taken as a unit from the Aa state to the AA state. For simplicity, however, we may substitute "x" for "y" in the above relation, because it seems presumable that a similar probability of reversion may exist constantly all through the plant life. Such a plant will have the following constitution in regard to the generative tissue:

$$x(AA) + (1-x)[x(AA) + (1-x)(Aa)].$$

As the result of self-pollination, the progeny of such a parent plant will show the constitution:

$$x(AA) + (1-x)[\tfrac{1}{4}(1+x)^2(AA) + \tfrac{1}{2}(1-x^2)(Aa) + \tfrac{1}{4}(1-x)^2(aa)].$$

Applying arbitrary values to "x" in this formula, we shall get numerical relations among segregates. In Table V the results of such calculation are compared with results obtained by the experiments in 1913–1915. Thus we may find the average partial homozygosity of heterozygotes around 4 to 6 per cent., the average partial fertility of sterile plants being, as was already shown, *ca.* 4 per cent.

TABLE V

CALCULATIONS ON DATA OF TABLE IV

x %	(AA+Aa)	aa	AA	Aa
4 %	77.88 %	22.12 %	38.74 %	61.53 %
5 %	78.57	21.43	39.69	60.31
6 %	79.24	20.76	40.89	59.11
Observation, No. of Inds.	9,918	2,643	94	135
Percentage	78.96 %	21.04 %	41.05 %	58.95 %

It has also been noticed that the sterility concerned is associated with an abnormality represented by the behavior of chlorophyll at the ripening of seeds. While, at the ripening season, the chlorophyll in the fertile sections of the mosaic forms turns to yellow just as in ordinary fertile plants, the chlorophyll in the sterile sections still

remains green. The fertile spikelets occurring in a small number on the otherwise sterile panicle appear on ripening as yellow spots scattered among green spikelets; the plants with both sterile and fertile panicles appear in the fall also as mosaic forms with green and yellow leaves. This feature of the sterile plants is in direct contrast to the behavior of the mosaic plants with the variegated and the entirely green leaves studied by De Vries and Correns.

The observations in the foregoing pages seem to parallel those made by the authors cited at the beginning of this paper. In the present investigation, however, there was observed also the transformation of allelomorphs in the opposite direction, that is, the transformation of the dominant allelomorph into the recessive allelomorph, something scarcely mentioned in the investigations referred to above. The observations in this regard were in brief as follows.

In the first place, the spontaneous occurrence of segregating families was observed again among the descendants of the families which had proved in the experiments already described to be constantly fertile. This suggests, just as did the occurrence in Family *A* and Family *B* in 1912, the probability of the AA cell changing into the Aa cell.

In the second place, a constant tendency of the dominant allelomorph to be transformed into the recessive allelomorph was observed in certain strains. In 1913, special attention was paid to such segregating families in which the excess of recessive segregates over the theoretical expectation was particularly high. Although, as already noted, the variation among the segregating families in 1913 with regard to the deviations from the recessive proportion might possibly have arisen from experimental errors associated with a certain probability of allelomorphic reversion from recessive to dominant, yet it was deemed not impossible that the very considerable excess of recessives exhibited by certain families might be caused by other reasons. This point was seemingly de-

cided by the experiment made with Family *B*80 in 1913 (Table VI), since in this family there was noticed a constant tendency toward the allelomorphic transformation under consideration.

TABLE VI

THE SEGREGATION OF FAMILY *B*/80 AND ITS DESCENDANTS

Year	No. of Families	Parent-plants	No. of Individuals	Fertile Plants	Sterile Plants	Recessives	Deviation of Recessives	P. E.
1913....	1	Fertile	99	69	30	30.30%	+ 5.30%	2.95%
1914....	10	"	1,020	727	293	28.73	+ 3.73	0.91
1915....	5	"	435	309	126	28.89	+ 3.89	1.40
1916....	98	"	11,013	7,832	3,181	28.88	+ 3.88	0.28
Total..	114	Fertile	12,567	8,937	3,630	28.89%	+ 3.89%	0.26%
1914 (*a*).	16[1]	Sterile	199	147	52	23.62%	− 1.38%	2.04%
1914 (*b*).	13[1]	Sterile	100	5	95	95.00%	+70.00%	2.92%
1915....	59[2]	"	548	32	516	94.16	+69.16	1.25
1916....	120[2]	"	1,436	99	1,337	93.11	+68.11	0.77
Total..	192[3]	Sterile	2,084	136	1,948	93.47%	+68.47%	0.64%

[1] Derived from the family in 1913, *i. e.*, Family *B*/80.
[2] Derived from the group (*b*) in 1914.
[3] Excluding the group (*a*) in 1914.

In Table VI there is beside the *ca.* 4 per cent. excess of recessives in the families derived from fertile parents, a remarkable excess of recessives in the families descended from the sterile parents in the group (*b*) in 1914. The sterile plant of this type could not be distinguished from those which, as was shown in Table IV, gave segregating families with an excess of dominants in the intensity of the partial fertility as well as in the behavior of chlorophyll at the ripening of the seeds. Consequently, it may be presumed that although these two types of sterile plants have the same genetical constitution originally, the dominant allelomorphs resulting from the reversion of their recessive allelomorphs are of different stabilities in the dominant state; that is, in the first type of sterile plants such dominant allelomorphs are very easily re-transformed into the recessive state, while in the second type the corresponding dominant allelomorphs tend to remain in the reverted condition.

Corresponding to the excess of recessive segregates, a deficiency of dominant homozygotes among dominant segregates was also noticed. Among 153 families derived from fertile plants in the experiment above mentioned, 40 families were uniformly fertile, the remaining 113 families showing segregation. The former, therefore, is 26.14 per cent. of the total number of families, and shows 7.19 per cent deficiency from the theoretically expected percentage, 33.33 per cent., the probable error being ± 2.68 per cent.

In conclusion it may be stated that the allelomorphs concerned in this investigation are probably subject to reversible transformations, and that the probable frequency of the allelomorphic transformation may be practically constant in a certain strain, and possibly may be different in different strains. As to the conditions under which such allelomorphic transformations take place, nothing is yet certain except that these conditions are of a hereditary nature. The manner in which different intensities of allelomorphic transformations are inherited will be the subject of further investigation.

A word may be added here regarding the conception of dominance and recessiveness. Bateson's theory of "presence and absence of factors" is sometimes understood in the sense that the dominant allelomorph is regarded as due to the real presence of an hereditary material unit which is absent in the recessive allelomorph. Such a conception is not in full accordance with the idea of the reversible transformability of allelomorphs as described in this investigation. There is another possibility of the nature of allelomorphs. The dominant and the recessive allelomorphs may be supposed to represent two alternative conditions or phases of a single hereditary substance, somewhat resembling the chemical conception of polymerization. Consequently, the interchangeability between the dominant and recessive allelomorphs is not improbable theoretically.

BUSSEY INSTITUTION,
August 26, 1917

NOTES AND LITERATURE

MUTATIONS IN DROSOPHILA BUSCKII COQ.[1]

Two mutations in eye color have appeared in my cultures of *Drosophila busckii.* These mutations are of especial interest in that, as far as the writer has been able to learn, they are the first that have been recorded in this species. This is the eighth species of *Drosophila* in which mutations have been recorded, the other seven being *ampelophila, repleta, confusa, tripunctata, virilis, obscura,* and *similis.*

The eye mutant which is brighter than normal has been called "red" and the other which is darker than normal has been called "chocolate." The normal eye of this species is darker than that of *Drosophila ampelophila.* The mutant red corresponds very closely to the normal eye color of *ampelophila* except that it is slightly brighter. Ridgeway's "scarlet" (Plate I, color number 5, Ridgeway's Color Standards and Nomenclature, 1912) corresponds most nearly to the eye color of this mutant. In the red eye the central fleck shows as a small round point, while in the normal *busckii* eye, it appears larger and less definite in shape. The red eye darkens with age and closely approaches the normal eye in color, but at its darkest stage it can be distinguished from the normal in that it is less translucent. The chocolate eye is an opaque brown and presents none of the shiny appearance of the normal eye. The central fleck is invisible in newly emerged flies, but becomes more or less distinct as the fly ages. With age the color approaches normal, but always remains slightly darker. Flies over forty-eight hours old so nearly approach normal that they are difficult to distinguish. A newly emerged chocolate corresponds most nearly to Ridgeway's "chestnut brown" (Plate XIV, color number 11, tone m).

The mutation red eye was first observed in November, 1916, and it seems probable that the original mutation occurred somewhat earlier and was overlooked as several red-eyed flies, both males and females, were obtained from this cross. The original stock had been collected about a month earlier in a tomato patch

[1] From the Zoological Laboratory of Indiana University, Contribution No. 157.

near Bloomington, Indiana, and had been bred in the laboratory for two generations.

The mutation chocolate eye was first observed in December, 1916, and here again the original mutation had probably been overlooked as in the cross where the mutation was first observed, several chocolate males and females appeared. The original stock in which this mutation appeared had been bred in the laboratory for three generations and was collected in the same tomato patch where the original red eye stock was collected. The stock in which the red eye appeared was collected on September 19, 1916, and the stock in which the chocolate eye appeared was collected September 14 of the same year and these stocks had been bred as two separate strains when the mutations appeared.

THE GENETIC BEHAVIOR OF RED EYE

Some of the first observed red males were mated to virgin red females and the offspring of this cross were all red flies. This red stock has been kept going for several generations and has given all typical reds. Both red males and red females were crossed with normal wild flies and in F_1 of each cross nothing but normal flies were found. Table I gives the results of the F_2 of these crosses.

TABLE I

F_2 OF RED × WILD CROSS

Red ♀ × Wild ♂

Culture Number	Type of Mating	Normal		Red		Total Normal	Total Red
		♂	♀	♂	♀		
332	1 pair	317	322	125	119	639	244
333	" "	305	313	109	107	618	216
334	" "	275	282	100	98	557	198
335	" "	326	299	97	101	625	198
336	Mass	557	552	112	150	1,109	262
Total		1,780	1,768	543	575	3,548	1,118

Red ♂ × Wild ♀

337	1 pair	340	326	101	105	666	206
338	" "	269	293	79	88	562	167
339	" "	231	253	71	83	484	154
340	" "	244	250	59	86	494	145
341	Mass	469	466	87	100	935	187
342	"	265	228	90	93	493	183
Total		1,818	1,816	487	555	3,634	1,042
Grand total		3,598	3,584	1,030	1,130	7,182	2,160

It can be readily seen that the red eye acts as a non-sex-linked recessive character with the red class falling a little short. Shortage of the mutational class frequently occurs. It seems to make no difference whether the red male or red female is used in the cross. In most cases when mass cultures were made, the expected 3 to 1 ratio was less nearly approximated. The ratio of all the flies examined in the F_2 was 3.32 normals to 1 red.

The Genetic Behavior of Chocolate Eye

A pure stock of chocolate was obtained by mating some of the first observed chocolate males to their virgin chocolate sisters. This stock has bred true for several generations but since the eye changes so rapidly to a color approximating normal, in stocks where the flies are allowed to become more than twenty-four hours old, all gradations between the typical chocolate and normal will be found. The chocolate males and females were bred to wild normals and the F_1 flies were all normal. The results of the F_2 of these crosses are shown in Table II.

TABLE II

F_2 of Chocolate × Wild Cross

Chocolate ♀ × Wild ♂

Culture Number	Type of Mating	Normal		Chocolate		Total Normal	Total Chocolate
		♂	♀	♂	♀		
402	1 pair	263	212	43	95	475	138
403	" "	154	146	49	60	300	109
405	" "	223	181	57	109	404	166
406	Mass	529	468	67	136	997	203
Total		1,169	1,007	216	400	2,176	616

Chocolate ♂ × Wild ♀

408	1 pair	257	252	58	66	509	124
409	" "	238	242	45	61	480	106
411	" "	343	312	58	82	655	140
412	" "	83	67	17	17	150	34
413	Mass	812	667	78	161	1,479	239
414	"	426	440	106	133	866	239
Total		2,159	1,980	362	520	4,139	882
Grand total		3,328	2,987	578	920	6,315	1,498

Chocolate eye also acts as a non-sex-linked recessive character with the chocolate class falling considerably below the expected

number. The number of chocolate males, especially, falls low. The totals seem to indicate that this low number of chocolate males in comparison to the number of normal males is partially due to the fact that some of the chocolate males have been called normal, for in practically all of the matings, the number of normal males exceeds the number of normal females. This is unusual, for the writer has examined large numbers of wild *Drosophila busckii* and in a large majority of the cases the number of females has been equal to or greater than the number of males. So it may be that the males approximate the normal color more rapidly than the females and since the flies were examined only once a day, some of the chocolate males were mistaken for normals. Some counts were made, examining the flies twice a day, to test this supposition and they indicated that better ratios could be obtained in this manner. But since the work was completed before this was realized, the difference to be obtained by twice-a-day counts was not thought to be of sufficient importance to require the repetition of the experiments. Also in these matings, the relative number of chocolates was lower where mass cultures were made. The ratio for all of the F_2 flies examined was 4.21 normals to 1 chocolate.

THE GENETIC BEHAVIOR OF RED AND CHOCOLATE WHEN THEY ARE MATED TOGETHER

Red males were crossed to chocolate females and red females to chocolate males and in the F_1 of each cross nothing but normal flies appeared. The results of the F_2 of these crosses are given in Table III.

Here again the number of normal males is considerably above the number of normal females. This could be explained as before, that some of the chocolate males have been mistaken for normals, thus increasing the normal class and decreasing the chocolate class. Since the number in the classes of red and chocolate each fell low in their respective crosses to wild, we can expect the number in these classes to be low in this cross. Taking this fact into consideration, the ratio can be considered a 1:2:1 ratio and gives indication of linkage between the two characters. No red-chocolate double recessives were found, therefore the two mutations may be interpretated as being located in the same chromosome.

TABLE III

F_2 OF RED × CHOCOLATE CROSS

Red ♂ × Chocolate ♀

Culture Number	Type of Mating	Normal		Red		Chocolate		Total Normal	Total Red	Total Chocolate
		♂	♀	♂	♀	♂	♀			
450.....	1 pair	237	206	95	122	86	92	443	217	178
451.....	" "	149	132	70	56	56	48	281	126	104
452.....	" "	212	184	78	108	73	83	396	186	156
453.....	" "	207	186	86	85	70	93	393	171	163
455.....	" "	246	238	114	129	88	106	484	243	194
456.....	Mass	373	360	149	153	85	109	733	302	194
Total.....	.....	1,424	1,306	592	653	458	531	2,730	1,245	989

Red ♀ × Chocolate ♂

457.....	1 pair	232	178	21	22	41	72	410	43	113
458.....	" "	173	163	62	68	48	59	336	130	107
459.....	" "	214	178	79	66	66	60	392	145	126
460.....	" "	186	179	93	99	50	58	365	192	108
461.....	" "	243	249	94	117	90	94	492	211	184
462.....	Mass	370	326	121	156	90	129	696	277	219
Total.....	.....	1,418	1,273	470	528	385	472	2,691	998	857
Grand total...	.....	2,842	2,579	1,062	1,181	843	1,003	5,421	2,243	1,846

Since mutations have occurred in eight species of *Drosophila* it seems probable that mutations may be found in all the members of this genus. As to the frequency of mutations, there may be individual variation. The writer's own experience would indicate that mutations occur less frequently in *busckii* than in *ampelophila,* for, during the same period in which the two *busckii* mutations were found, a smaller number of *ampelophila* were examined less critically and six mutations were found.

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SINGING MICE

IN November, 1916, Mr. B. S. York, of Ann Arbor, brought to me a "singing" house mouse that had been captured in his home. This mouse had been heard by members of his family for several weeks, especially late at night and early in the morning. Arrangements were made to carry on breeding experiments with it but it lived only two weeks.

Singing mice have been recorded in a number of publications dating back many years. In 1912 Coburn[1] reported some work he had done with a female singer captured in December, 1911. This individual when mated with an ordinary mouse gave birth to five litters (thirty-three young). None of these were singers and no singers appeared in either the second or third generations. Two other singing mice were described by Coburn in 1913.[2] One was caught in the home of an Italian family in November, 1912, and the other was taken by a farmer in Michigan in March, 1913. Both of these were females.

The Ann Arbor specimen that was brought to me also proved on dissection to be a female. Her song was similar to that reported by Coburn as follows:

> The sound is best described as a rapid whole-toned trill involving the tones *c* and *d*. . . . The quality of the tone resembled somewhat that of a fife or flute, but each tone ended with a slight throaty click.

In every case the song could be heard at least 15 or 20 feet away.

Many causes have been proposed for the presence of this ability to sing such as pregnancy, a diseased condition of the lungs or vocal cords, a parasitized liver, etc. There were no embryos or young in the Ann Arbor specimen and Dr. George R. LaRue was unable to find any parasites that could have induced the singing.

It has been suggested that since all of the singers captured thus far have been females, this characteristic may be sex-linked and due to some structural modification of the vocal apparatus.

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[1] Coburn, C. A., *Journ. Animal Behavior*, Vol. 2, 1912, pp. 364–366.
[2] Coburn C. A., *Journ. Animal Behavior*, Vol. 3, 1913, p. 388.

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THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG. II

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5. *Interaction of Nucleoplasm and Cytoplasm*

There are phenomena that occur during the growth period that suggest how masses of cytoplasm that are differentiated both morphologically and physiologically may arise in the cortical layer of the insect egg. It has been suggested that "most of the differentiations of the egg cytoplasm have arisen during the ovarian history of the egg and as a result of the interaction of nucleus and cytoplasm; . . .,"[27] and with this we fully agree, but our problem is to determine the nature of this interaction and in what ways it may take place.

During every mitosis there is a more or less thorough mixing that involves the chromatin as well as other nuclear constituents, since chromatin-diminution is a normal histological process. Interchanges between nucleus and cytoplasm, therefore, occur during the two multiplication periods that precede the formation of oocytes. Abundant opportunity is thus offered for factors in the chromosomes to exert an influence upon the cell as a whole. A similar and probably even greater discharge of chromatic

[27] Conklin, 1916, "Heredity and Environment," New York.

and other nuclear substances into the cytoplasm occurs during the maturation divisions of the egg, but this period may be neglected in this connection, since the organization with which we are concerned is already established before maturation takes place. Even when the nuclear membrane is intact, substances undoubtedly pass in and out of the nucleus much as they do through the cell membrane, and as in the latter, the nuclear membrane may change in permeability at different times, these changes being due to chemical processes taking place within the nucleus or in the cytoplasm. Such changes occur more often during periods of cell activity than at other times and thus we should expect pronounced interaction throughout the growth period of the oocytes.

Besides gradual, and for the most part invisible, interchanges of this sort there may be actual transference of visible masses of chromatin from the nucleus to the cytoplasm. These chromatin granules that escape into the cytoplasm have been called "chromidia" and are supposed to play a part in cytoplasmic differentiation.

A peculiar process of interchange by means of secondary nuclei is exhibited by certain insects, especially Hymenoptera.[28] This process has been studied most carefully in the carpenter ant, *Componotus herculeanus* var. *pennsylvanica* (Fig. 11). At an early stage in the growth of the oocyte small vesicles containing a few granules of chromatin appear near the oocyte nuclei. These "secondary nuclei" appear to arise as buds from the primary nucleus, but no one has yet actually observed their formation in this way. It has also been suggested that they may be epithelial cells that have invaded the oocyte, but this seems very improbable. The writer has reached the conclusion that they consist of nuclear materials that have been given off into the cytoplasm and have there become enclosed by membranes which give them a nuclear-like appearance. As the oocyte increases

[28] Blockmann, 1886, *Festsch. nat.-med. Verein zu Heidelberg;* Buchner, 1913, *Biol. Centrlbl.*, Bd. 33; Hegner, 1915, *Journ. Morph.*, Vol. 26.

in size the secondary nuclei increase in number until they entirely surround the primary nucleus, forming several layers. When the oocyte has nearly reached its full growth they begin to migrate from the group near the anterior end of the oocyte and become scattered through-

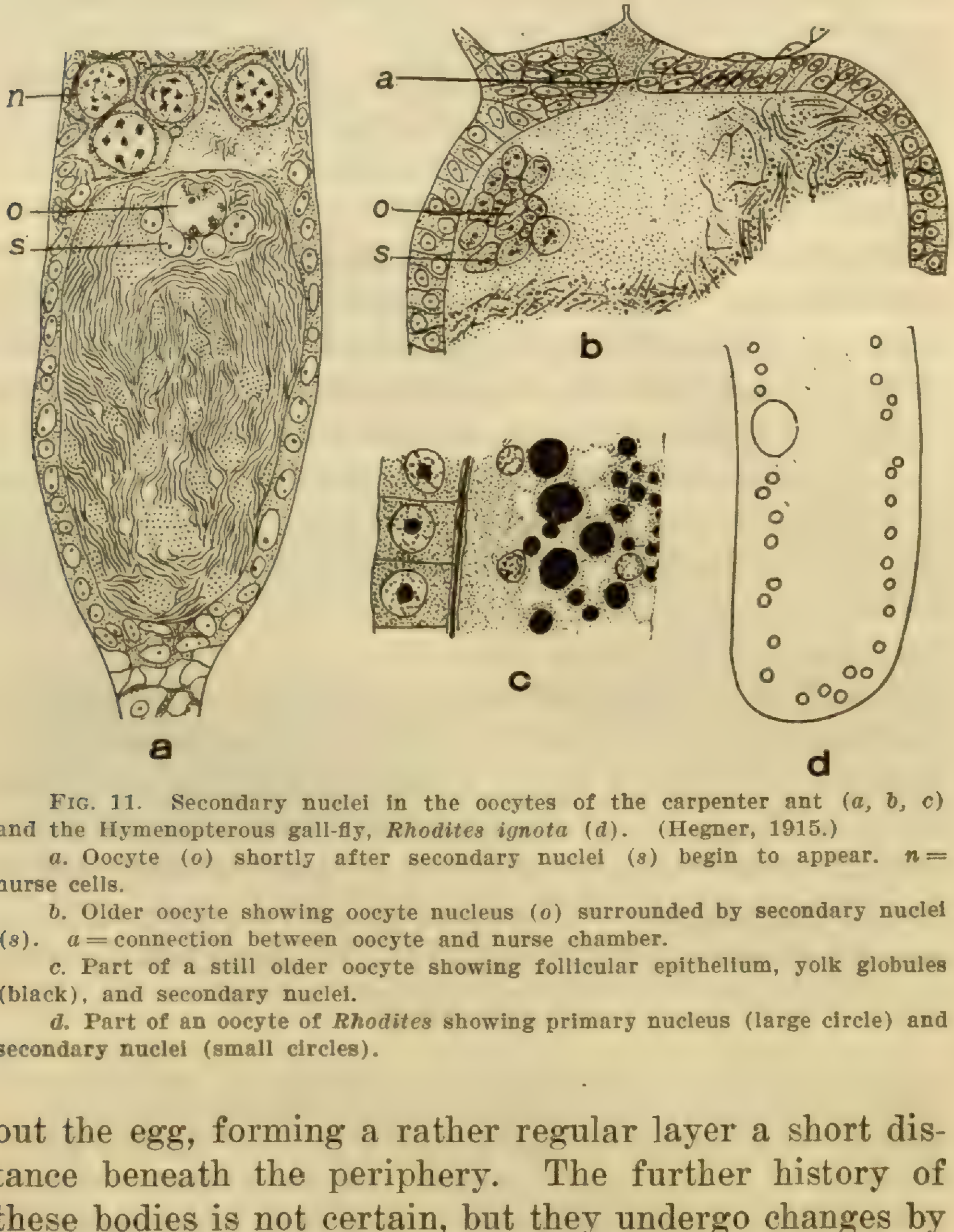

FIG. 11. Secondary nuclei in the oocytes of the carpenter ant (*a*, *b*, *c*) and the Hymenopterous gall-fly, *Rhodites ignota* (*d*). (Hegner, 1915.)

a. Oocyte (*o*) shortly after secondary nuclei (*s*) begin to appear. *n* = nurse cells.

b. Older oocyte showing oocyte nucleus (*o*) surrounded by secondary nuclei (*s*). *a* = connection between oocyte and nurse chamber.

c. Part of a still older oocyte showing follicular epithelium, yolk globules (black), and secondary nuclei.

d. Part of an oocyte of *Rhodites* showing primary nucleus (large circle) and secondary nuclei (small circles).

out the egg, forming a rather regular layer a short distance beneath the periphery. The further history of these bodies is not certain, but they undergo changes by which they lose their identity, since they can not be found in fully grown eggs. Their function is likewise problematical. They may take part in the formation of germline determinants which probably occur in the eggs of

this ant;[29] they may aid in changing the substances furnished by the nurse cells into material available for the embryo;[30] or they may have something to do with the formation of yolk.[31] It is also possible that they may control differentiation in the peripheral layer of cytoplasm and thus provide a method of nuclear control of the organization of the egg. The last hypothesis may be objected to on the grounds that the secondary nuclei appear to be irregularly distributed and that they are known to occur in only a few species of insects.

Another possible way in which the initial organization of the insect egg may arise is through the activities of mitochondria. The rather constant presence of these bodies in the cytoplasm of almost all types of cells indicates that they may be of considerable importance in the process of differentiation. If they take part in the genesis of egg organization they then may play the rôle attributed to them by certain investigators of being the cytoplasmic bearers of hereditary factors corresponding in this respect to the nuclear bodies of similar function, the chromosomes.

The most striking differentiation in the cytoplasm of the insect egg is that which involves the germ-line determinants. As stated above, we do not know for certain in any case the origin of the peculiar cytoplasmic mass that contains these determinants, but a number of hypotheses have been suggested. In *Miastor*, for example, the following is offered to account for the appearance of the "pole-plasm" in the fully developed oocyte.[32]

It may be distinguished from the rest of the egg contents by its position at the posterior end and because of its affinity for certain dyes. It appears shortly before the maturation division is initiated, but no transition stages have been discovered—it has been either present or entirely absent in the preparations thus far studied. If we consider the history of this substance from the formation of the primordial germ

[29] Hegner, 1914, *Journ. Morph.*, Vol. 26.
[30] Marshall, 1907, *Zeit. wiss. Zool.*, Bd. 86.
[31] Loyez, 1908, *C. R. Assoc. Anat.*, 10 Reunion, Marseille.
[32] Hegner, 1914, "Germ-Cell Cycle in Animals," New York.

cell to the growth period of the oocytes produced by this primordial germ cell, we may conclude that at the time the multiplication period ends the pole-plasm has become equally distributed among the sixty-four oogonia. Then ensues the growth period during which the pole-plasm can not be distinguished. Later, however, just before maturation, pole-plasm substance reappears which is equal in amount to that contained in the primordial germ cell of the preceding generation or to that contained in all of the sixty-four oogonia which descended from that primordial germ cell. That is, the pole-plasm of the oocyte under discussion has in some way increased until its mass is sixty-four times as great as that of the oogonium before the growth period began. How this increase has taken place can only be conjectured. The pole-plasm in the oogonium may have produced new material of its own kind either by the division of its constituent particles or by the influence of its presence.

The influence of a specialized mass of cytoplasm upon the chromatin is very well illustrated by the inhibition of chromatin-diminution in *Miastor* and *Ascaris*. In *Miastor* nuclear division is normal until at the four-cell stage one nucleus reaches the pole-plasm at the posterior end (Fig. 12, *a*, IV.). During the succeeding mitosis this nucleus, which is apparently under the control of the pole-plasm, does not undergo chromatin-diminution, whereas the other three do. One of the daughter nuclei resulting from the division of this undiminished nucleus remains entirely within the pole-plasm and is cut off from the rest of the egg with this specialized mass of cytoplasm as the primordial germ cell (Fig. 12, *b*). This nucleus always retains the full amount of chromatin; but its sister nucleus, which remains in the egg and is thus separated from the direct influence of the pole-plasm, undergoes diminution at the next mitosis.

A similar segregation of specialized cytoplasm in the primordial germ cells occurs also in certain other insects and in copepods, but no diminution process has yet been discovered in them. In *Ascaris*, where chromatin-diminution was first reported,[33] there is evidently a segregation of germinal cytoplasm at each cleavage division up to the sixteen-cell stage, when it is all confined in one cell, the

[33] Boveri, 1887, *Anat. Anz.*, Bd. 2.

primordial germ cell. This cytoplasm, which is not visibly different from the rest, as in *Miastor,* appears to inhibit diminution in every nucleus that comes within its

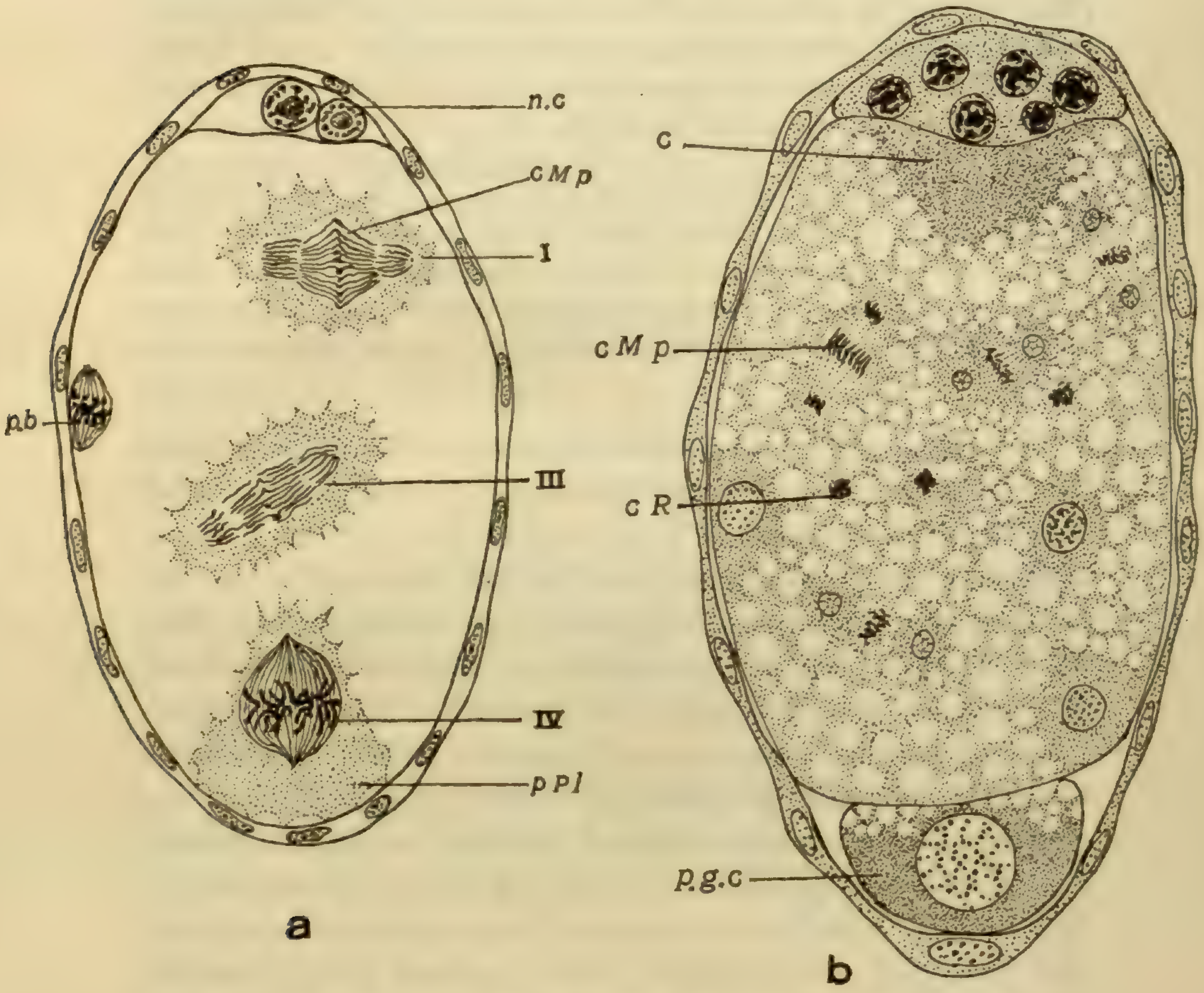

FIG. 12. *a.* Longitudinal section through an egg of *Miastor* showing chromatin-diminution in nuclei I. and III. but not in nucleus IV. which has come under the influence of the pole-plasm (*p Pl*). Nucleus II. does not appear in this section. *cMp* = chromatin that is cast off into cytoplasm. *nc* = nurse cells. *pb* = polar body. (Kahle, 1908.)

b. Longitudinal section through an egg of *Miastor,* showing primordial germ cell (*p.g.c.*), nuclei undergoing chromatin-diminution (*cMp*), and the remains of chromatin cast out into the cytoplasm (*cR*), *c* = cytoplasm elaborated by nurse cells above. (Hegner, 1914.)

immediate influence as indicated by experimental studies on dispermic and centrifuged eggs.[34] In this respect it resembles the pole-plasm of *Miastor.*

[34] Boveri, 1910, *Arch. Ent.-mech.,* Bd. 30; Boveri, 1910, *Festschr. R. Hertwig.,* Bd. 3.

6. *Mendelian Factors and Cytoplasmic Organization*

The central biological problem of the present time is the method of evolution, and a knowledge of the mechanism of heredity has long been recognized as necessary for its solution. The results derived from breeding experiments with the fruit fly, *Drosophila ampelophila*, have dominated the field of genetics for the past five years, but although of very great interest and importance, their evolutionary significance is not yet certain. To be of primary value from this viewpoint it is necessary to prove that new species may arise by means of Mendelian characters (mutations) such as white eye, miniature wing, club wing, etc. Since no one has ever been able to define satisfactorily what a species really is and hence what characters should be considered of specific value, this is a difficult problem.

The definitions given by two of our foremost authorities, one a systematist and the other a geneticist, are as follows: The systematist writes:[35]

> Forms of animals which present distinct assemblages of characters, in form, color and arrangement of parts under natural conditions, which are recognizable from descriptions and figures, should receive distinctive names and be catalogued, provided, of course, that the assemblage of characters includes all ontogenetic changes. If, in the examination of abundant material from different natural environments, we find these characters fairly constant, the forms may properly be called species; if not, varieties or races.

The geneticist writes:[36]

> Species may thus be distinguished by peculiarities of form, of number, of geometrical arrangement, of chemical constitution and properties, of sexual differentiation, of development and of many other properties. In any one or in several of these features together, species may be found distinguished from other species.

The mutations that have appeared in *Drosophila* do not become recognizable until a late stage in the life history of the individual, and are about the last characters

[35] Williston, 1908, AMER. NAT., Vol. 42.
[36] Bateson, 1913, "Problems of Genetics."

to appear in the individual development. They for the most part affect the size and shape of the wings, the size, shape and color of the eyes, and the color of the body.

If a systematist were asked whether these new races of *Drosophila* are comparable to wild species, he would not hesitate for a moment. He would call them all one species. If he were asked why, he would say, I think, "These races differ only in one or two striking points, while in a hundred other respects they are identical even to the minutest details." He would add, that as large a group of wild species of flies would show on the whole the reverse relations, viz., they would differ in nearly every detail and be identical in only a few points.[37]

This point of view seems justified, since the foremost dipterologist in this country, a man who has named over one thousand species and genera, mostly of flies, says regarding the results of certain experiments carried on with *Drosophila* by one of his colleagues.[38]

But I think it is absolutely certain—and I speak as an entomologist fairly familiar with flies—that it would be impossible to produce species of his sports even though they were bred for a thousand years.[39]

In talking over this species question with one who has had considerable experience in systematic work[40] it became clear that although as a rule only a few of the more conspicuously contrasting characters are selected for descriptive purposes, as a matter of fact the individuals of different species are often different in practically every morphological characteristic. One who is very familiar with these species will realize these differences at once, although many of them are of such a nature that they can not be described so that any one else will recognize them. There seems to be no difficulty, however, in finding numerous describable contrasting characters in *Drosophila*, since at least fifty-nine are included in the descriptions of two recently named species[41] that were selected

[37] Morgan, 1916, "Critique of the Theory of Evolution."
[38] Dr. F. E. Lutz.
[39] Williston, 1908, AMER. NAT., Vol. 42.
[40] Dr. Alexander G. Ruthven.
[41] Sturtevant, 1916, *Annals Ent. Soc. Amer.*, Vol. 9.

at random, *D. superba* and *D. projectans*, and these characters relate to almost every part of the body. Many other differences would probably also be found between the physiological processes and general activities of the adults and between the morphological and physiological characteristics of the embryos, larvæ and pupæ of the two species if they were compared from these standpoints. It has been shown that the factor for the character club wing affects not only the character that gives this mutation its name, but also other characters, for example the presence or absence of a pair of spines on the sides of the thorax, these being always absent when the factor for club wing is present.[42] It is possible that the combination of a number of such factors as that for club wing would ultimately satisfy the requirements of systematic entomologists and that new species could then be made up in the laboratory. Such mutations might therefore be of evolutionary value. If, however, these mutations fail to furnish characters of specific rank, or characters that may *lead* to the formation of new species, we must conclude that they are not of evolutionary significance, and look elsewhere for the factors that are responsible for specific characters and that may undergo changes which lead to transmutation.

Factors of this sort may lie in the chromosomes or in the cytoplasm, but they are probably the results of interaction between chromosomes and cytoplasm. As pointed out above, interaction of this sort has abundant opportunity to operate during the germ-cell cycle. The cytoplasmic differentiations resulting from the metabolic processes that culminate in the formation of an egg ready to undergo maturation are very striking in the case of insects, as indicated by observations and experiments on the eggs of chrysomelid beetles, and there seems to be no valid reason why the eggs of these beetles are different in their type and complexity of organization, both morphological and physiological, from those of *Drosophila;* for while we do

[42] Morgan, Sturtevant, Muller, and Bridges, 1915, "Mechanism of Mendelian Heredity."

not know much about the growth of the egg and embryological development of this genus of flies, we do know that these processes in certain other flies resemble those of beetles.

If the Mendelian factors are located in the chromosomes, it is evident that they may exert an influence upon the entire contents of the egg, (1) during the mitotic divisions of the oogonia, (2) during the so-called resting stages of the oogonia, and (3) during the growth of the oocytes. It is also clear that all of the factors carried by the chromosomes have an equal opportunity to interact with the cytoplasm and not alone those that remain within the egg after the elimination of chromosomes during maturation. The adult, however, that develops from the egg, whether fertilized or unfertilized, exhibits only those detailed characteristics whose genetic factors are supposed to be located in the chromosomes remaining in the egg after maturation, or in those that are brought in by the sperm. This seems to indicate that none of these factors has any permanent influence upon the egg organization during the growth of the oocyte and until maturation is completed.

It seems impossible to ignore the chromosomes or even to locate the principal factors of heredity in any other cell bodies. It may therefore be necessary to reconstruct our ideas of chromosome architecture and thereby abandon the theory that these bodies consist of a linear series of factorial determiners for certain ferments and of nothing else. It may be possible to separate our hypothetical factors into two groups, (1) those responsible for such characteristics as the polarity, bilaterality and "pattern" of the egg, and (2) those that control mutations that appear at a late period in the life history like those that are so abundant in *Drosophila*. Perhaps the latter may be anchored to the chromosomes as has recently been suggested;[43] the main portion of the chromosomes might then represent the foundation for the factors responsible for the organization of the egg and the attached masses of

[43] Goldschmidt, 1917, *Genetics*, Vol. 2.

ferments might constitute the factors responsible for the modification of embryonic, larval and adult characters—factors such as have been employed for experimental breeding purposes by most geneticists. According to this hypothesis it would probably be necessary to consider the main portions of each chromosome as sufficient for the production of an entire organism. The fact that the group of factors carried by any one chromosome in *Drosophila* controls characters that are not restricted to any definite part of the body gives weight to this assumption.

Most geneticists are accustomed to deal with adult characters only, and on this account pay very little or no attention to the eggs, embryos and larvæ of the species they are experimenting with. But the eggs, embryos and larvæ contain all the factors for these adult characters, both those that are realized and those that are inhibited either by internal or external causes, and they may exhibit characters that make it possible to separate different lines although the adults may be indistinguishable. Furthermore, taxonomists have long recognized the value of embryonic characters as an aid in determining species.

We should always be careful to distinguish between the parts of the egg that are of hereditary significance and those that are not. Thus the shell or chorion of the silkworm egg has been discussed under the heading of "cytoplasmic inheritance," whereas it is not a vital part of the egg, but, being secreted by the epithelium of the ovarian tube, is a well-defined characteristic of the adult female and its coloration, which follows the laws of Mendelian inheritance,[44] is controlled by maternal factors.

Such fundamental characteristics as polarity, symmetry, and pattern, which are so clearly exhibited by the eggs of insects and certain other animals, are much more difficult to study than adult characters and are probably not so easily modified. If any or all of them are carried over from one generation to another in the cytoplasm we have then a real instance of cytoplasmic inheritance. Even if this is the case the chromosomes doubtless exert

[44] Toyama, 1913, *Journ. of Genetics*, Vol. 2.

an influence upon the cytoplasm during the oogonial and growth periods of the egg, and a study of the genesis of cytoplasmic organization may lead to data that will help us solve this difficult problem.

If the polarity of the oocyte when recognizable is not inherited, *i. e.*, if it is not transmitted to the primordial germ cells by the egg, and retained by the oogonia, it must arise *de novo* just before or during the growth period. One observer[45] has found that in certain beetles the position of the spindle remains, resulting from the differential divisions that precede the formation of the oocyte, indicates the polarity of the ultimate organism, but he does not tell us how this "polarité predifferentielle" is brought about. In all insects the end of the egg directed toward the head of the mother becomes the anterior end of the offspring. This is also the pole of the egg lying next to the nurse cells or that is closest to the nurse-cell chamber. This relation between oocyte and nurse-cells may be the determining factor in the polarity of these eggs and, if so, would indicate that polarity here is due to environment. How this relation could influence the polarity may be explained by means of axial gradients of metabolism, such gradients in this case being produced by greater external stimulation at the end near the nurse-cell chamber where nutritive substances are elaborated and added to the oocyte. By this theory of metabolic gradients, differentiation along an antero-posterior axis can be accounted for and further differentiations of a morphological and physiological nature would result from "chemical transportative correlation between the different parts."[46]

We should not lose sight of the fact, however, that these hypothecated physiological activities require protoplasm as a material basis and that their results depend upon the character of this protoplasm. If polarity is established at the stage suggested above, it follows a long series of nucleo-cytoplasmic interactions which have no doubt resulted in the differentiation and localization of numerous

[45] Govaerts, 1913, *Arch. Biol.*, Tome 28.

[46] Child, 1916, *Science*, Vol. 43.

kinds of cytoplasm. The appearance of a definite polarity might lead in some way to diffusion processes and the circulation of secretions resulting in further specializations and localizations. One stage seems to initiate the next stage in the series of processes that accompany the visible changes in the growth and development of the egg, and the character of these processes is of course due to the specificity of the protoplasm.

That the cytoplasm may exert a controlling influence upon the chromatin has been demonstrated in several instances. For example, we know that the chromatin-diminution processes during the early cleavage of both *Ascaris* and *Miastor* are controlled by the cytoplasm and that in these animals the germ-cell nuclei retain the full amount of chromatin because of the germ-cell cytoplasm they chance to encounter.

Probably the peculiar distribution of the chromosomes at certain stages in the life histories of certain aphids, phyloxerans, and Hymenoptera is also controlled by the cytoplasm. In the aphid, *Aphis saliceti*, the first maturation division is visibly differential both as regards the chromosomes and the cytoplasm.[47] The mitochondria congregate at one end of the dividing spermatocyte; this process is accompanied by a greater accumulation of cytoplasm at this end so that cell division results in one large cell containing all of the mitochondria and about two thirds of the cytoplasm, and one functionless small cell. The large cell also receives three chromosomes; the small cell only two.

The peculiar maturation divisions in the males of the honeybee[48] and hornet,[49] during which one ultimate spermatogonium gives rise to only one spermatozoon instead of the usual four, may also be the result of cytoplasmic control. The cytoplasm may likewise be responsible for the passing of a sex chromosome into the polar body during the maturation of the egg of certain aphids at the end of the summer season.[50] Such eggs must be fertilized

[47] Baehr, v, 1909, *Arch. für Zellf.*, Bd. 3.

[48] Meves, 1907, *Arch. mikr. Anat.*, Bd. 70.

[49] Meves and Duesberg, 1908, *Arch. mikr. Anat.*, Bd. 71.

[50] Morgan, 1909, *Journ. Exp. Zool.*, Vol. 7 .

before they will develop, and always produce males. Many other peculiarities in the behavior of chromosomes that have been reported from time to time may also be due to the influence of the environment (cytoplasm), and there seems to be no reason why factors carried by the chromosomes should not be affected by the cytoplasm as well as are entire chromosomes.

By the interaction of Mendelian factors with the cytoplasm during the germ-cell cycle, it is even possible to explain the fact that "crossing over" occurs in the females of *Drosophila,* but not in the males.[51] In the latter, the spermatocytes do not pass through a pronounced growth period, and hence there is comparatively little nucleo-cytoplasmic interaction, and since the cytoplasm carried by the sperm may be considered negligible, the factors borne by its chromosomes are not interfered with. In the female, however, there is ample opportunity for such interaction during the growth period, and factors at this time may be influenced by the cytoplasm or may influence the cytoplasm in such a way as to cause an irregular distribution of chromosomal factors.

To the writer the following conclusions seem justified. The insect egg at the time of maturation is a mosaic of differentiated cytoplasmic areas predetermined to develop into definite parts of the embryo. This organization has resulted from the interaction of nucleus and cytoplasm during the germ-cell cycle. Such interaction is taking place at all times, but is visible only when such processes as the protrusion of chromidia or chromatin-diminution occur. The many cases of cytoplasmic control over chromatin behavior, and the apparent failure of the factors for the characters commonly used by geneticists to influence the egg organization, indicate the importance of more careful studies of the genesis of this organization. The importance of such studies is emphasized by the possibility that they may help toward the solution of the problem of the method of evolution.

[51] Morgan, Sturtevant, Muller, and Bridges, 1915, "Mechanism of Mendelian Heredity."

NEW FACTS AND VIEWS CONCERNING THE OCCURRENCE OF A SEXUAL PROCESS IN THE MYXOSPORIDIAN LIFE CYCLE.[1]

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THE classic observations of Balbiani, Bütschli and Thélohan on the myxosporidian development do not include the occurrence of a sexual process which comprehends the forming of a syncaryon in the life history of this protozoan group. Doflein (1898 and 1901) suggested *two* places in the life cycle of the myxosporidian where a caryogamy might probably take place. In the next period of investigations on myxosporidia the occurrence of this sexual process was stated by various authors, but they differ widely in the conception of the place in the life cycle in which the copulation occurs. Mercier, Awerinzew,

[1] For a clear understanding of the question under discussion, it is necessary to have a uniform nomenclature and to discard all terms which are only of historical value. Noyaux du sporoplasma, noyaux du germe, noyaux sporoplasmiques, germnuclei, Amöboidkeimkerne were to be discarded and gametonuclei, noyaux des gamètes, Gametenkerne were to be used. Instead of sporoplasma, sporoplasm or amöboidkeim, only such expressions as gametes, gametes and Gameten are admissible. Identical and adequate terms are capsulogenous cell, cellule capsulogène, Polkapselzelle. Valve cells, cellules valvaires and Schalenzelle should be used for those cells which form the membrane of each single spore; cellule d'enveloppe, envelope cells, and Hüllzellen should be used for those cells which form the membrane of the pansporoblast. In cases where only the nuclei of these cells are present, the terms noyaux d'enveloppe, envelope nuclei, Hüllenzellenkerne should be substituted. If the cellular origin of the pansporoblast membrane is not ascertained, envelope, membrane d'enveloppe or Hülle may be used. The terms Restkern, residual nucleus are misleading. Somatic residual nuclei or somatische Restkerne should be used, if the definition on p. 679 holds true. Here a new name would be of great value to eliminate the wrong analogies created by Doflein (1898, p. 309). The term "reduction nuclei" is only justified if the numerical reduction of chromosomes has been ascertained.

Auerbach and Parisi try to show that a real syncaryon formation takes place at the *onset* of spore formation. Other authors, Keysselitz, Schroeder and Auerbach, believe that only a plasmogamy can be pointed out at the beginning of spore formation, and that the union of the nuclei is effected either in the fully developed spore or in the young animal leaving the spore. The difference between these last two conceptions is theoretically without significance because the main part of copulation—the union of the nuclei—takes place *at the onset of the new life cycle of the myxosporidian.* Therefore it was of the utmost importance for decisive proof of this fact to find the copulation of the two gametonuclei inside the fully developed spore or in the young myxosporidian. Schroeder, 1909, observed the copulation of the two gametonuclei in the spore; Auerbach, 1907 and 1910, found young animals of *Myxidium bergense* with one nucleus. I was able to demonstrate young *Chloromyxum leydigi* which were experimentally produced by placing the two-nucleated spores on gall plates (Erdmann, 1911). Here, after a treatment with intestinal secretions of the host the young animals leave the spore. They are at first binucleate, later uninucleate. In my recent work, finished in 1913, which did not appear until 1917 in consequence of the war, I figured these young animals after fixation and staining. Also, Davis, 1915, though with some reserve, presents young *Sphærospora dimorpha* which have left the spore and show the fusion of their two nuclei. Later the separation of the syncaryon into its vegetative and generative components takes place. Georgevitch, 1914, presents the development of the young animal in *Henneguya gigantea* in—as it seems—changed peculiar conditions. The spore is still inside of the cyst of the big "tumor-forming" tissue-parasite. The binucleate form becomes uninuclear and then the usual vegetative multiplication of the nuclei begins, which leads up to a *renewed* spore formation *inside* of the tumor cyst. In *Chloromyxum leydigi,* a gall-bladder parasite, no such

complicated process takes place. As the young uninucleated forms develop we see an animal with three nuclei, all of the same size. Later multiplication of these vegetative nuclei and the formation of big syncytial masses occur. The plasmatic bodies of these vegetative animals contain two kinds of round corpuscles, "Reservekörper" and "Farbträger." In my publication in the *Archiv für Protistenkunde*, 1917, I give proof that the "Reservekörper" consists mostly of glycogen and I point out that the glycogenous contents are used up during spore formation. The vegetative animal can multiply either by division or by forming small vegetative gemmules (Erdmann, 1911). The fact that inside the animal vegetative propagative bodies can arise, was verified by Davis, 1915, pp. 354–355, in *Sphærospora dimorpha*.

Before the onset of spore formation a differentiation in the syncytial masses of *Chloromyxum leydigi* begins. We can distinguish parts in which the nuclei multiply and other parts where only the vegetative nuclei are seen widely scattered in the protoplasm. I called the first-mentioned areas "islands" (Erdmann, 1911) because in the living animal they rise above the surface of the vegetative plasmatic body. They are distinguished by their pale color and in stained preparations by their large number of small nuclei. At first all the nuclei in these islands are of the *same* size. Two nuclei with small cytoplasmic bodies approach each other and each cell divides up into a small and a big cell. The two small cells draw out in length and surround the two big ones, in this manner separating them from the other cells in the island. This quadruple group, two big cells and two small ones, is the starting point for the formation of the whole spore. The two big cells are gametocytes. These two gametocytes divide and form two gametes and two other cells which after a further division give rise to four cells—these four cells are the four capsulogenous cells. The whole spore contains, therefore, eight cells—four capsulogenous cells, two gametes and two cells which form the spore membrane. (Fig. 1.)

I mentioned before that the glycogen which was found in the vegetative body is used up in spore formation. The membrane of the spore, the polar threads and the darkly staining structureless lumps, which have been seen by all authors inside the sporoblast, consist of glycogen and stain as well by chromatin as by specific glycogen stains. These lumps have been considered as "reduction nuclei" by various authors. They have also been called "Restkerne" or "residual nuclei." It may be emphasized for later discussion that they are glycogenous and not chromatic in *Chloromyxum leydigi*.

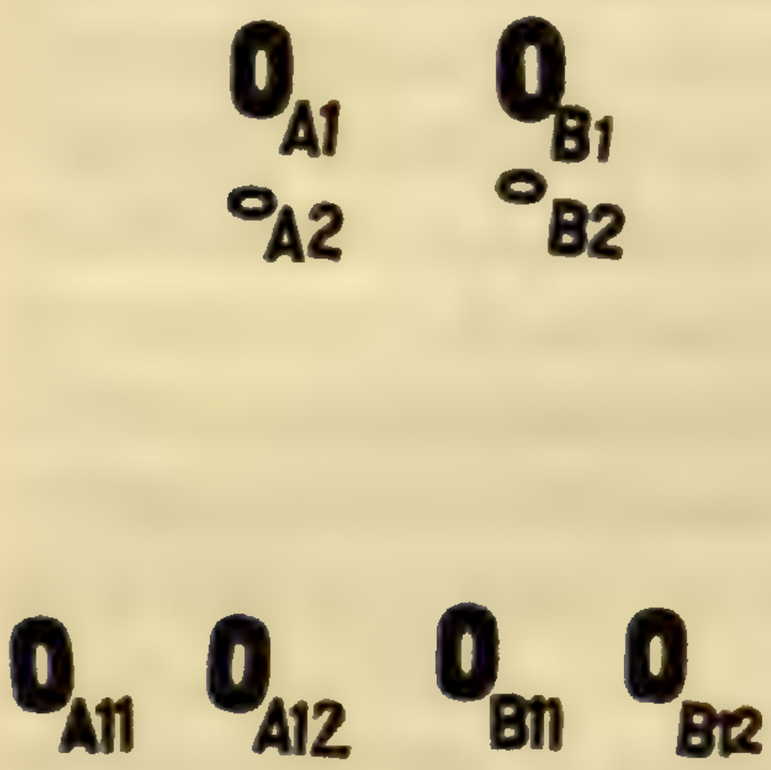

FIG. 1. · *Chloromyxum leydigi:* A_2 and B_2 are the spore membrane forming cells; A_{11} and B_{12} are the gametes. A_{121} A_{122}; B_{111} B_{112} are the capsulogenous cells.

Our knowledge of the sexual process in the myxosporidian life cycle since the investigations of Keysselitz, Schroeder, and others has been completed by recent authors: Georgevitch, 1914, Davis, 1915 and Mavor, 1916. The best proof that *no* reduction takes place in the spore is given by Georgevitch (1914) and Davis (1915), who have been able to study the number of chromosomes in their specimens before and after spore formation. Both authors agree that the number of chromosomes is not changed before and after this phenomenon. Davis figures six chromosomes in *Sphærospora dimorpha* and Georgevitch, investigating *Henneguya gigantea*, finds eight. These two facts: *first, that the number of chromosomes is not changed in spore formation* and, *second, that the so-called "reduction nuclei" inside the spore are really glycogenous* bodies which are used up in forming the membrane of the spore, polar threads, and spore membrane of *Chloromyxum leydigi*, positively prove that the real reduction must occur at a different place in the life cycle of *Sphærospora, Chloro-*

myxum, and *Henneguya*. The reduction consisting in the transformation of a diploid nucleus into a haploid or of a tetraploid one into a diploid can only occur at the beginning of the new life cycle *after* or *before* the union of the gametonuclei.

The further facts which Davis, 1915, presents in *Sphærospora dimorpha* also tend to show that reduction could only take place at the above outlined place. The gametonuclei of this myxosporidian fuse together after it leaves the spore and form by one subsequent division *two* nuclei. One of these nuclei gives rise to *all* the cells of the later sporogenous body. The other of these two nuclei, distinguished by its size and structure, is the vegetative nucleus of the animal, the somatic "Restkern." *No other nuclei should be called "Restkerne" except when they represent the nucleus or nuclei of the vegetative myxosporidian body, which does not play a part in spore formation.* Such "Restkern" or "Rest-

TABLE I

Author	Species	Occurrence of Somatic Residual Nuclei in Sensu-stricto, See Definition, p. 679	Occurrence of Hüllzellen Envelope Cells, Pansporoblast-membrane Forming Cells or Only Their Nuclei	Number of Valve Cells. They Are Division Products of Gametocytes
1. Awerinzew.	*Myxidium* sp.	One	—	2 for each spore
2. Auerbach...	*Myxidium bergense*	None (p. 26)	—	"
3. Davis.....	*Sphærospora dimorpha*	One, seldom two	—	"
4. Awerinzew.	*Ceratomyxa drepanopsettæ*	Two	—	"
5. Mavor.....	*Ceratomyxa acadiensis*	Two	—	"
6. Erdmann...	*Chloromyxum leydigi*	Many	—	"
7. Davis.....	*Sphærospora dimorpha*, polysporous form	Many	—	"
8. Auerbach...	*Myxidium bergense*, polysporous form	None (It may be that Auerbach has observed in his mono-, di- and polysporous forms only the propogative parts of the complete animal.)	—	"
9. Parisi.....	*Sphærospora caudata*	No facts mentioned	Two	"

kerne,'' the origin and fate of which agree with this definition, have been described by Awerinzew in *Ceratomyxa drepanopsettæ,* and in *Myxidium* sp., by Davis in *Sphærospora caudata,* and by Mavor in *Ceratomyxa acadiensis.*

In the disporous form, *Sphærospora dimorpha,* two spores are found in the whole animal and the sporogenous body finally contains twelve cells—half of this number forms one spore (Fig. 2). These twelve cells are all

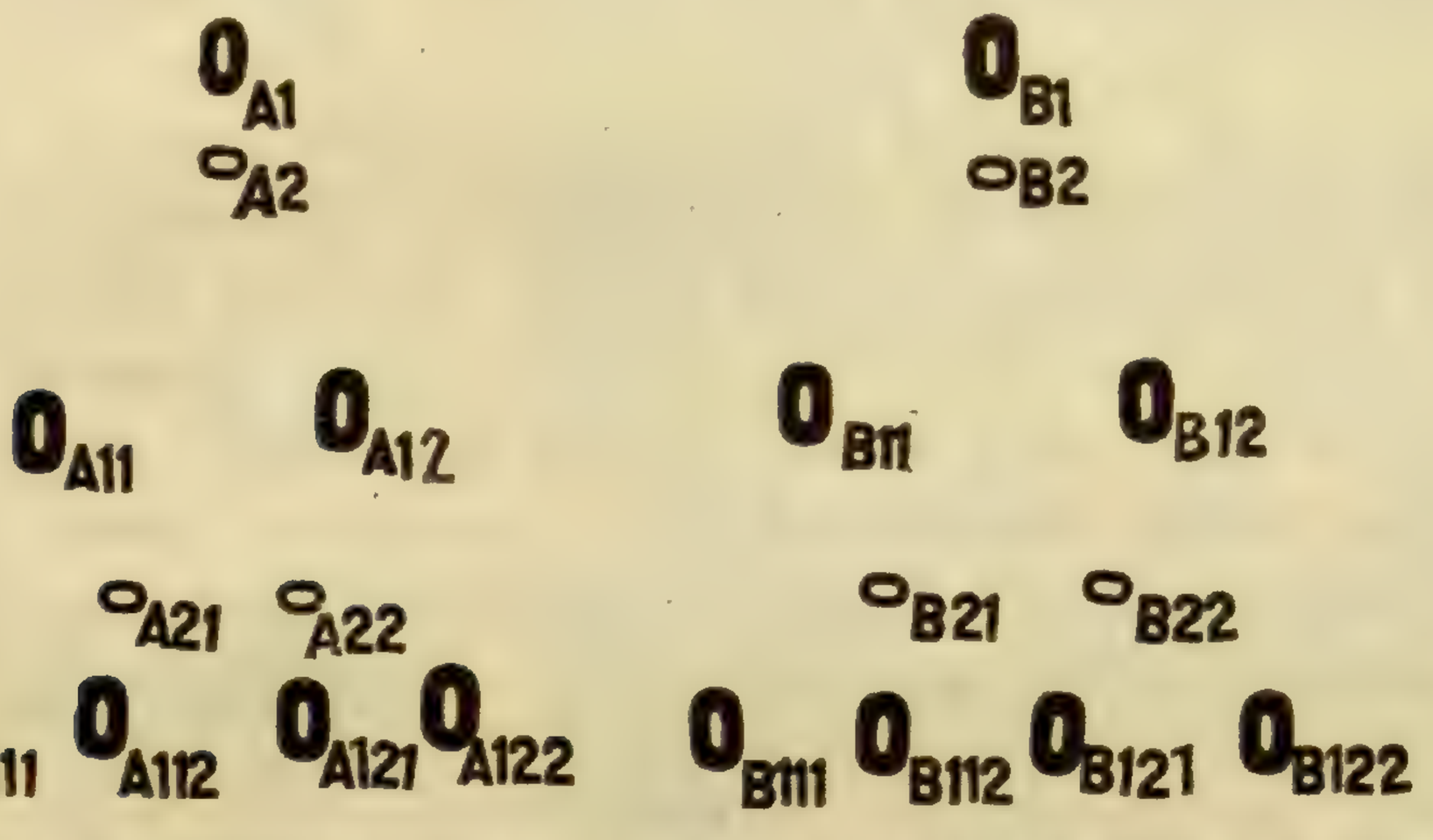

FIG. 2. *Sphærospora dimorpha* (disporous form): A_{21} A_{22} and B_{21} B_{22} are the membrane-forming cells for each spore. A_{111} A_{122}; B_{111} B_{122} are the two gametes in each spore. A_{112} A_{121}; B_{112} B_{121} are the two capsulogenous cells in each spore.

division products of the *one* nucleus, the *sister* nucleus of which is the somatic ''Restkern.'' The cells which form the spore membrane of each spore, lying independently in the vegetative body, arise by a very *late* division. Chromatic lumps which could be considered as ''reduction nuclei'' are lacking. It is easy to imagine why they are absent. *Sphærospora dimorpha* lives in the urinary bladder and has therefore a metabolism which may not afford opportunity for an abundant glycogen formation. Also, as mentioned before, no reduction of the chromosomes takes place. These facts could be easily ascertained in consequence of the large size of the cells and chromosomes. Awerinzew's description of spore formation in *Ceratomyxa drepanopsettæ* and Mavor's of *Ceratomyxa acadiensis* have many features in common, but Awerin-

zew's presentation differs from that of the other author in one important point. The cells *A* and *B* in Fig. 2 are said to be in *Ceratomyxa drepanopsettæ* the products of a fusion of two cells. The reader has but to change the lettering of *A* in *AC* and of *B* in *BC* to see that the two copulæ undergo changes identical with the two single cells in *Sphærospora dimorpha*. Except this one point of difference—the beginning of spore formation—we note that the late division of the membrane-forming cell, the identical number of cells in each spore and the independence of each spore in the myxosporidian body characterize both species of *Ceratomyxa* (Awerinzew and Mavor) and *Sphærospora* (Davis). It appears highly improbable that in two different species of *Ceratomyxidæ* the basis of spore formation should be a copula (Awerinzew) or an univalent nucleus (Mavor).

Summarizing the known description of spore formation of those myxosporidia, in which *each spore is formed independently of the other in the somatic body*, and where no pansporoblast exists, we can demonstrate the following uniform features in all investigated forms.

1. Six cells or nuclei are used for the formation of each spore, when two polar capsules are present; eight, when four polar capsules are present.

2. The cells which form the spore membrane have a similar origin and are distinguished by the independence in which these cells develop as compared with the other constituents of the spore. Their mother cell is lying in a resting stage till the division of the gametocyte is finished, as described by Davis for *Sphærospora dimorpha*, and by Awerinzew for *Ceratomyxa drepanopsettæ*.

In *Myxidium* sp., where, according to the investigations of Awerinzew, either one, two or three spores are lying independently in the myxosporidian body, there is a very late division of the cell, the divisional products of which form the spore membrane as recorded by this author (Fig. 3). Here the one gametocyte divides into two cells, one of which by a late division gives rise to the two-

spore membrane-forming cells, the other forms the two capsulogenous cells and two gametes. We intentionally avoid, in Fig. 3, calling the two chromatin lumps, which the author himself (p. 202) has called "überflüssiges Chromatin" (degenerierende Kerne), nuclei and do not add them as such in the diagram as Auerbach, 1912, p. 28, did when discussing Awerinzew's investigations. We consider these chromatin lumps only as formations probably of glycogenous nature and as being used during membrane formation.

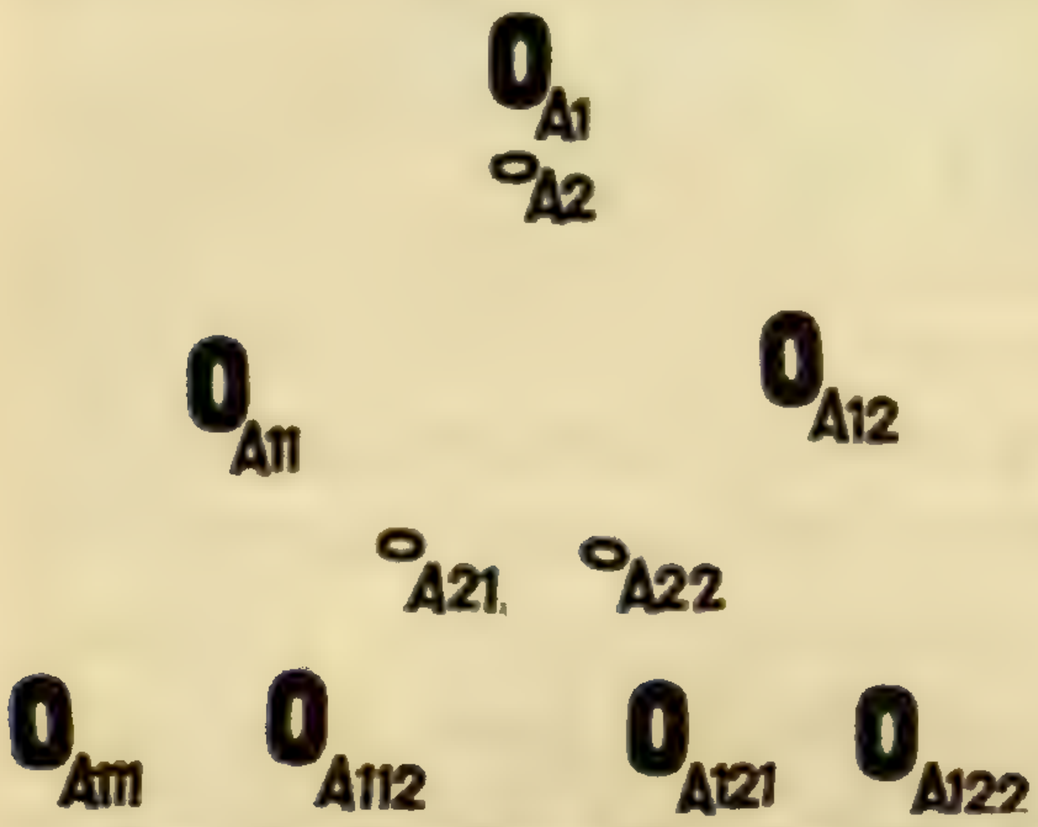

FIG. 3. *Myxidium* sp.: A_{21} and A_{22} are the spore membrane-forming cells (valve cells). A_{111} A_{122} are the gametes. A_{112} and A_{121} are the capsulogenous cells.

In Fig. 4 Auerbach's conception (Type I) of how the spore formation is effected in *Myxidium bergense* is represented. Auerbach believes, as stated before, that either a plasmogamy (Type I) or a real copulation (Type II) may be at the basis of spore formation. We will not discuss, for the present, how the bigger and smaller cells which are seen at the beginning of spore formation, arise. The latter divides once and the two divisional products form the spore-membrane-forming cells. The other cell divides twice to give rise to two gametes and two capsulogenous cells. The author does not especially mention the order in which these cells divide,

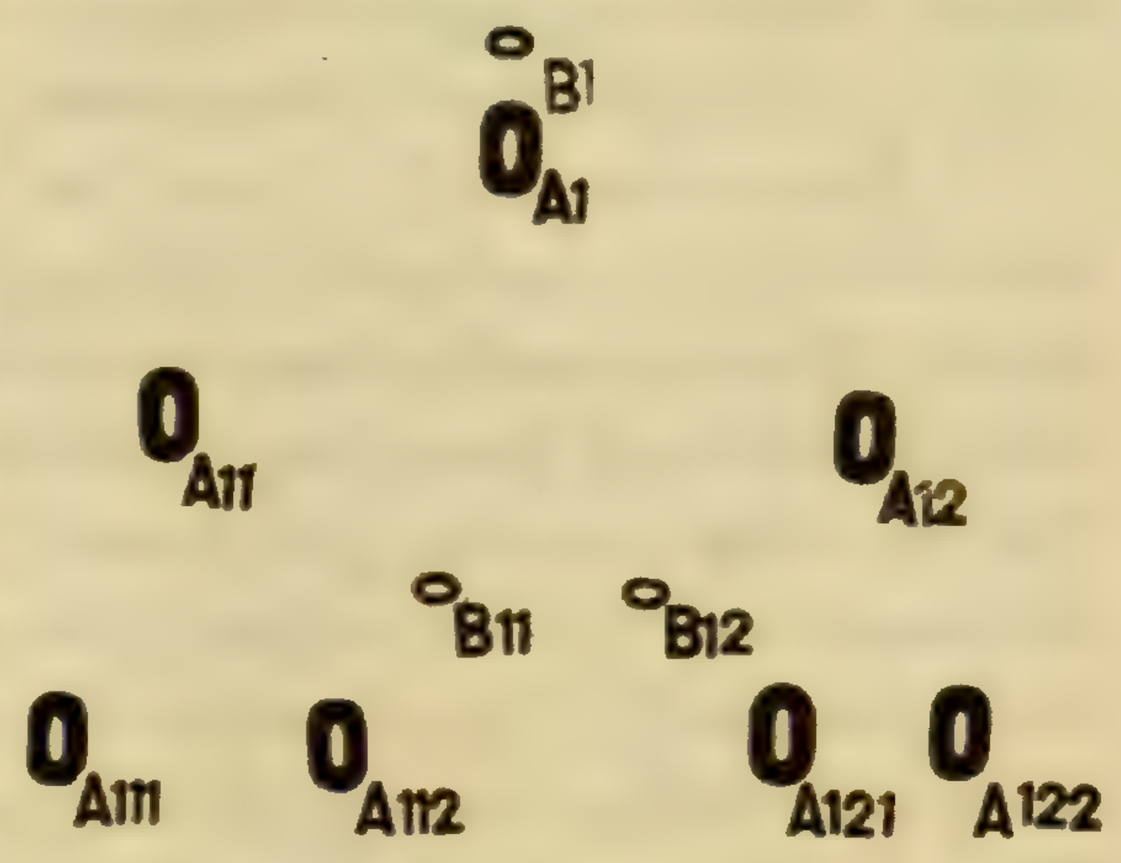

FIG. 4. *Myxidium bergense* (Auerbach Type I); B_{11} and B_{12} are the spore membrane-forming cells (valve cells). A_{111} and A_{122} are the two gametes. A_{112} and A_{121} are the capsulogenous cells.

but nothing in his report contradicts the supposition that in *Myxidium* sp. and in *Myxidium bergense* there is a close analogy with Davis's and Awerinzew's observations. He also observes the elimination of chromatin and "eventuell Bildung von Restkernen" (p. 24). These are not incorporated in Fig. 4 for the same reasons I pointed out for *Myxidium* sp.

My observations in *Chloromyxum leydigi* show, furthermore, that the cells which form the valves of the spore do not play a part in the development of the final contents of the spore, but are here in this form (comp. Fig. 1) the products of the first division of each gametocyte. In the case of *Chloromyxum leydigi two* gametocytes form by one division the two spore-membrane-forming cells; in the three other species, *Myxidium* sp. (probably *Myxidium bergense*), *Ceratomyxa drepanopsettæ* and *Sphærospora dimorpha, one* gametocyte forms the one cell, the division products of which are transformed into the valves of the spore. But in all four species these cells have the sole purpose of forming the spore membrane.

After surveying the Figs. 1, 2 and 3, and having compared them, I have no doubt that the origin of the spore-membrane-forming cells is identical in the so-called monosporous and disporous forms. Advancing one step farther and taking into consideration those forms in which two gametocytes form the cells inside each spore (Fig. 1)' we notice that in dealing with the origin and the position in the development of the spore, we have to add nothing. The spore-membrane-forming cells are distinguished by their early segregation from the gametocytes and their non-entering into the series of those cells which are included in the spore. The only difference is that these cells do not divide further; if they did, we could easily construct the disporous type of *Sphærospora dimorpha* (Fig. 2). The same holds true for those species which form two spores in one pansporoblast (Fig. 4) and where two gametocytes are observed at the basis of spore formation (Keysselitz, Schroeder).

If we conceive these two cells in question (A_2 and B_2) which form in *Myxobolus pfeifferi* (Keysselitz) the pansporoblast membrane to divide once more and the last division inside the spore to be suppressed, we could have the type of *Sphærospora dimorpha.*

According to Keysselitz, in *Myxobolus pfeifferi* each of the two gametocytes together with the small cell ap-

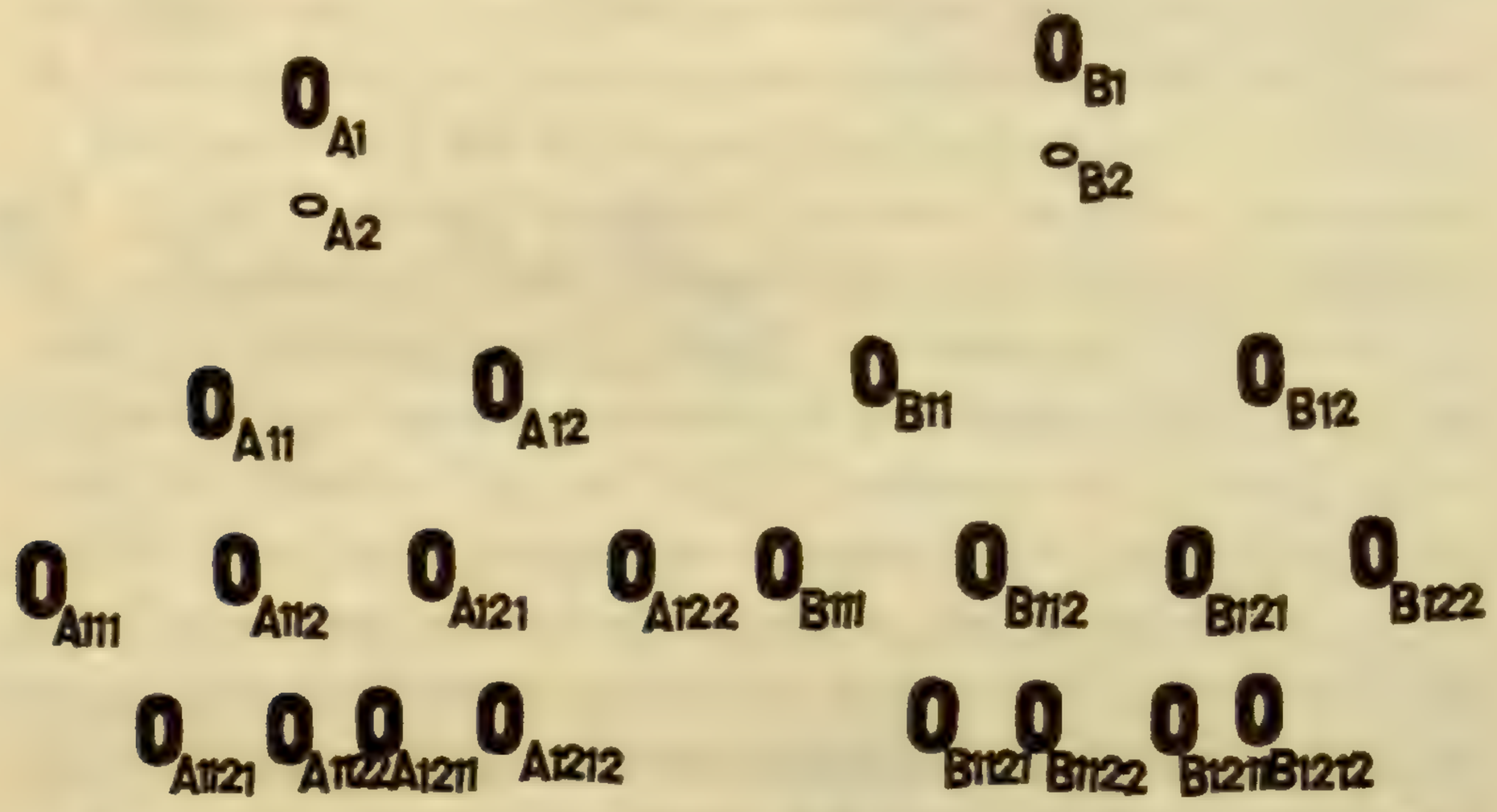

FIG. 5. General plan for polysporous disporoblastic forms. (Type Keysselitz.) A_2 and B_2 are the envelope-forming cells (pansporoblast-forming cells). A_{111} and A_{122}; B_{111} and B_{122} are the two gametes in each spore. The products of the fourth division form valve cells A_{1121}, A_{1211}, B_{1121}, B_{1212}, and capsulogenous cells A_{1122}, A_{1211}, B_{1122}, B_{1211}.

proach each other and each divides up until six cells have arisen. Thus we have in all cases 14 cells, two of which have a different divisional capacity, for they stop dividing and the big cells form all the other cells which at the end compose the two spores. Their later fate is indicated in Fig. 5 and, though this does not concern us in this discussion, we should like to emphasize the fact that two gametes are always present in a certain stage of spore development. We are convinced that the cells A_2 and B_2 represent genetically in the pansporoblastic forms that cell formation which in all monosporous, disporous and polysporous species gives rise to the spore membrane itself (Figs. 1 to 4). These cells or their nuclei were observed by Keysselitz, Parisi, Lo Giudice, Auerbach (*Henneguya psorospermica*), Georgevitch, and with cer-

tain restrictions by Mercier and Schroeder. This cell couple (A_2 and B_2) should be called envelope cells or envelope cells nuclei when they do not fulfill their destin (Schroeder, probably Mercier). Even in those cases in which a pansporoblast membrane had not been discovered it might have been either overlooked or have been in evidence at the beginning of pansporoblast formation before the valves of each single spore had been developed. Later these take up the function of the Hüllzellen which make their retrogressive development plausible. These Hüllzellenkerne are neither Restkerne, nor reduction nuclei, nor somative residual nuclei. The term for residual nuclei of somatic nature (see definition, p. 679) has already been disposed of in *monosporous* and *disporous* forms and must be used in the same way in polysporous forms. In all forms which are polysporous and have many singly developing spores, the whole vegetative body which is not used up in spore formation has somatic "restkerne" or residual nuclei. As I pointed out in *Chloromyxum leydigi* the vegetative animal may die after spore formation together with the "restkerne." In this form the vegetative animal may prolong its life by forming internal buds, if it has reached a considerable size before and during spore formation. In all polysporous forms with pansporoblast, *i. e.*, the disporoblastic forms, we have to be very careful when applying the name of somatic residual nuclei. In those species which are not tissue parasites and sometimes have cystlike formations which are surrounded by gelatinous envelopes, we may find somatic residual nuclei, because it seems improbable that the whole vegetative body is used up for spore formation. I believe this to be the case in *Sphæromyxum sabrazesi* and *Sphærospora caudata.* Where no residual vegetative nuclei were observed, the investigators may not have studied the whole animal, but only the propagative parts of it which have left the vegetative body (Parisi, Fig. 3). Keeping this point in mind, later investigators may discover somatic residual nuclei in de-

generating stages analogous to those found in the monosporous, disporous and the non-disporoblastic polysporous forms, among the debris of the dying animals inside the gall and urinary bladder, as I have shown in *Chloromyxum leydigi*. In the so-called "tumor-forming" disporoblastic polysporous forms, no *facts* are known which show that somatic residual nuclei have been observed. The beginnings of cyst formation, however, have never been studied, and it is only at this stage that somatic residual nuclei may be seen, and not after the cyst is crowded with sporoblasts and spores. But even in the fully developed cysts there may be degenerating somatic residual nuclei which have escaped observation. The facts which Weissenberg found in *Glugea anomala* and *hertwigi*—two Microsporidia—seem to support my suggestion. But the Hüllzellen or Hüllzellenkerne of the myxosporidia are never identical with somatic nuclei. Their undisputed place in the development of the myxosporidia will soon be clear.

Before we proceed further in discussing the formation of the sporoblast membrane in those myxosporidian species in which the spores are formed in *pairs* inside one sporoblast, it may be recalled that several facts have been ascertained concerning the fully discussed spore-membrane formation in monosporous or disporous myxosporidian species: (1) The copulation of two gametes occurs during or after the two myxosporidia leave the spore. (2) No reduction takes place from the beginning of spore formation until the end, because the number of chromosomes remains the same (Davis and Georgevitch). (3) The darkly staining masses of "restkerne," "residual nuclei," "reduction nuclei" have been shown to be glycogenous and to be necessary for spore-membrane formation. (4) Membrane-forming cells or nuclei are set apart by different division intervals from the other cells of the sporoblast.

Now from the above given summary of the latest facts in monosporous or disporous forms, it is clear that they

are strictly *opposed* to all the views which maintain that a copulation or so-called syncaryon formation precedes spore formation. But they are all in accord with the investigations of all authors who have shown that there is no syncaryon formation, but merely a plasmogamy of two cells, without any copulation, at the onset of spore formation.

When I wrote the second part of my investigations on *Chloromyxum leydigi,* 1913, I pointed out that the facts which were presented by Auerbach, Mercier and Parisi, as proofs of the occurrence of a syncaryon formation just before the onset of spore formation, are not quite convincing. Their figures can easily be arranged in such a manner that the supposed syncaryon formation represents the division of gametocytes into a smaller cell, which in most all other known cases forms the membrane of the pansporoblast. (Compare Erdmann, 1917.) It is not necessary to repeat here the attempted revision and rearrangement of the figures of these authors. This same holds true for the syncaryon formation in *Myxidium bergense,* Type II (Auerbach) and *Ceratomyxa drepanopsettæ* (Awerinzew). We will take it for granted that our views are correct as long as no new *facts* ascertained on smears—not sections—compel us to change our opinion.

As mentioned above, all authors who have shown that no syncaryon formation occurs, but that a plasmogamy of two cells without any nuclear fusion occurs at the onset of spore formation, can agree with us that the sexual process is going on at the beginning of the new life cycle. Auerbach and Parisi do not convince us that the figures which represent the so-called caryogamy can not be considered as the dividing of the gametocyte in the two cells. In accordance with the facts and interpretations, Keysselitz, Schroeder, Lo Giudice, Erdmann, and Georgevitch uphold the view that *no* merging of two cells or two cell pairs takes place to form the couples of cells which are later considerd as a quadruple group of the growing spore. By comparing the series of figures of all those drawings

which are supposed to prove the merging of two cells, they can, as said before, be interpreted as the division of one cell into two. The larger of these cells, wrongly called macrogametocyfe according to the cell fusion theory, has divided and formed the cell wrongly called microgametocyte.

This "microgametocyte" and its division products after one division, or these "microgametocytes" in cases where two gametocytes are observed at the onset of spore formation (Keysselitz, Schroeder, and Erdmann) now form, according to all known investigations, the pansporoblast membrane. Just as we could point out in disporous forms the uniformity of the origin of the spore-membrane forming cells (Figs. 2, 3 and 4) so we can do the same for the pansporoblast membrane and its nuclei in the following forms: *Myxobolus pfeifferi* (Keysselitz), *Myxobolus ellipsoides* (Lo Giudice), *Sphæromyxa sabrazesi* (Schroeder), *Sphærospora caudata* (Parisi), *Henneguya gigantea* (Georgevitch), and *Henneguya psorospermica* (Auerbach); all following Fig. 5, provided we do not take into consideration the origin of the cells A, A_2 and B and B_2. Keysselitz and Schroeder's views, except one contradictory point, are exactly represented by Fig. 5, but there are differences mentioned by the other authors. Still we make the generalization that there is one and the same plan of spore formation in the pansporoblastic myxosporidia though we know that facts are reported which do not fit in with our view. *We hold the opinion that it is permissible to rearrange the observed facts, because all interpretations have been gained by piecing together and arranging facts according to the theoretical viewpoint of the authors.* No continued observation of spore formation in the living animal has been possible. Also we are allowed to add facts ascertained in other species if the authors have only considered sections and not smears. Sections are misleading because the whole quadruple group can not always be seen on the same section and the origin of the small cell from

the big cell can not be traced without doubt. Therefore, most investigators have lately used smears to get a fuller and more correct view of the origin of the different cells from each other. It is astonishing how scanty the details appear when one considers the formation of the quadruple group in Auerbach's, Lo Giudice's, Parisi's and Georgevitch's presentations. Mercier's Figs. 19–27, Plate 1; Auerbach's Figs. 8*a*–15, Plate 2; Lo Giudice's Figs. 29–34, Plate 1; Parisi's Figs. 13–18, Plate 16; and Georgevitch's Figs. 32–35, Plate 1, do not show each single step of this important process. Connecting stages are missing. Therefore, one is allowed to interpret differently *their* presented facts, as I have done in Fig. 5. In Table II

TABLE II

I	II
Myxobolus pfeifferi Mercier.	*Sphæromyxum sabrazesi* Schroeder.
Cell *A* (copula) forms all other 12 cells of the pansporoblast and the two "Hüllzellenkerne."	A_2 B_2 A_1 B_1 All cells divide up to form the 12 pansporoblastic cells and the two "Hüllzellenkerne."

Myxobolus ellipsoides Lo Guidice.	*Henneguya gigantea* Georgevitch.	*Henneguya psorospermica* Auerbach (Type I).
	A_2 A_{22} A_1 A_{12}	
	Cells A_2 and A_{22} do not form any of the other 12 cells of the pansporoblast.	

we can study the different opinions from the authors' point of view. Lo Giudice, Georgevitch, Auerbach, Keysselitz, and Schroeder are alike in interpreting that the two cells which develop into the pansporoblastic membrane or nuclei are separated very early from the other cells. They never intermingle with those cells inside the spore-membrane (except according to Schroeder). They can not, therefore, be microgametocytes and in consequence they have nothing whatsoever to do with a sexual phenomenon. This adds strong support to our view that

the sexual process is at the beginning of the new life cycle.

I do not wish to veil the great discrepancy between the conception of Mercier (Fig. 6) and the other authors

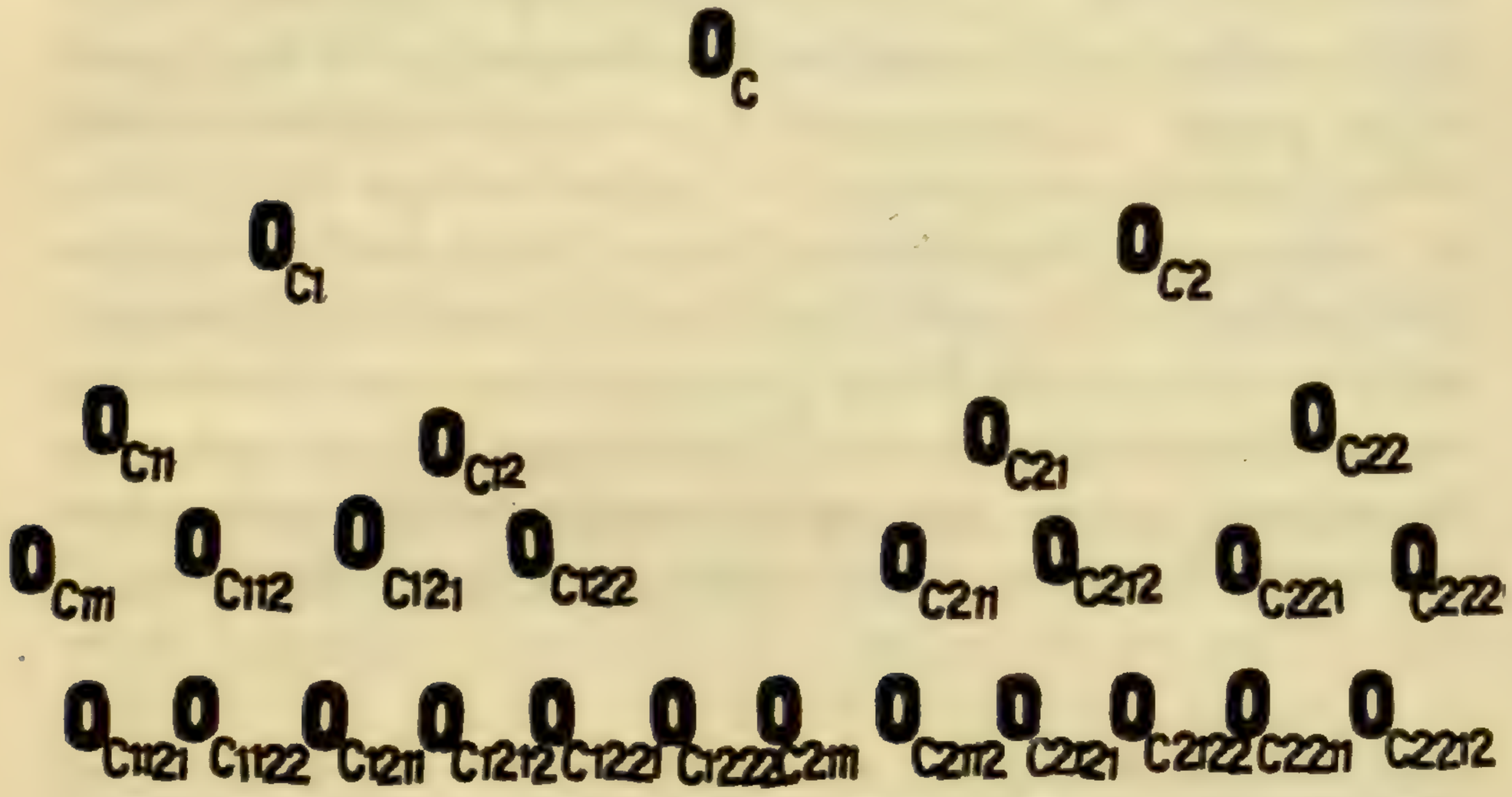

FIG. 6. *Myxobolus pfeifferi,* Mercier: C_{111} and C_{222} are the celles d'enveloppe of this author, which represent the false "Restkerne" or "reduction nuclei" of other authors. The products of the fourth division form, for each spore, the gametes, the valve cells and the capsulogenous cells.

mentioned. All cells are products of a copula, and there is no setting apart of the pansporoblast-membrane-forming cells or nuclei, though later they appear at the accustomed places between the two spores (Plate I, Figs. 31, 32). I shall not risk an interpretation, but think a new investigation on this same.subect might be very promising and result in the desired uniformity. I think it highly probable that in all pansporoblastic forms the spore development follows the Keysselitz-Schroeder interpretation: that two gametocytes form the basis of the spore formation. But even if one believes that the quadruple group is not formed by two but by one cell pair, the principal point is not changed. It is indifferent for the theoretical interpretation whether a segregation of the second cell pair from the first takes place, and both then form the quadruple group, or whether two cell pairs approach each other and form the quadruple group. The pædogamy is merely a closer one in the first case.

To summarize: In the observed myxosporidian species

with pansporoblast (exception, Mercier, *Myxobolus pfeifferi*), the first division products of the gametocyte or gametocytes form the pansporoblastic membrane or, if degenerated, its nuclei. This division is a heteropole division and forms highly chromatic small cells or nuclei which never intermingle with the cells inside the pansporoblastic membrane (exception, Schroeder).

On the basis of these facts, we need only state that the heteropole division has no connection whatsoever with a reduction division, as Keysselitz tentatively suggested. This conspicuous division produces the Hüllzellen or Hüllzellenkerne, and it may not be impossible that in the case of *Sphæromyxa sabrazesi* these small chromatin cells do not intermingle with the others and divide up, though the author mentions it on page 366. They originate, according to Schroeder's second interpretation, in the same manner as most authors describe, but divide together with the big cells until twelve cells are present in the pansporoblast; they then take their accustomed place inside the sporoblast membrane and are easily recognizable. This apparent exception merits further investigation.

We maintain our conclusion that the Hüllzellenkerne or the Hüllzellen are identical with the spore membrane forming cells of the non-disporoblastic polysporous forms. They have the following features in common: they are the first division products of the gametocytes; they do not intermingle with the other cells inside the spore; they form the envelope, in one case of the spore, in the other of the pansporoblast. They are neither somatic residual nuclei nor Restkerne nor reduction nuclei. They are cells which have a tendency to degenerate in some disporoblastic forms when their functions are taken up at an early period by the valve cells or spore-membrane-forming cells.

It remains, as the last part of our discussion, to deal fully with the significance of those darkly staining masses which have been described as "Reductionskerne" or "Restkerne" "*inside* the sporoblast." In the following table we give a short survey of the known facts.

A survey of Table III brings out clearly certain facts. When darkly staining masses are observed inside the

TABLE III

Author	Species	Occurrence of Chromatic Bodies Inside the Spore Interpreted as Reduction
1. Awerinzew	*Myxidium* spec.	Seldom distinct small nuclei, generally "Zwei ziemlich grosse Chromatinkügelchen" (p. 201).
2. Auerbach	*Myxidium bergense*	Diffusion of chromatin or formation of two "restkernartigen Gebilden" (p. 20).
3. Davis	*Sphærospora dimorpha*	Formation of round chromatic bodies.
4. Awerinzew	*Ceratomyxa drepanopsettæ*	Infiltration of chromatic small bodies into the cytoplasm before the spore membrane includes the gametocytes after the supposed copulation. Note here the later formation of spore membrane.
5. Mavor	*Ceratomyxa acadiensis*	Formation of round chromatic bodies at the onset of spore formation (Fig. 10) which are later resolved.
6. Erdmann	*Chloromyxum leydigi*	Formation of several large, deeply staining bodies which disappear after the spore membrane is formed.
7. Parisi	*Sphærospora caudata*	Formation of small, deeply staining bodies before the spore membrane includes the gametocytes after the supposed copulation (Fig. 15).
8. Davis	*Sphærospora dimorpha*, polysporous form	No diffused infiltration of chromatin observed, also no formation of round chromatic bodies.
9. Auerbach	*Myxidium bergense*, polysporous form	Formation of two "restkern artigen Gebilden" or diffusion of chromatin.

10. Keysselitz	*Myxobolus pfeifferi*	One to four round chromatic, deeply staining bodies, disappearing after the valves of the spores are formed (p. 261).
11. Mercier	*Myxobolus pfeifferi*	Diffusion of small chromatic bodies into the cytoplasm (Figs. 33, 34) after the sporoblast membrane is formed, also the same fact stated after the supposed copulation (Figs. 22, 23).
12. Lo Guidice	*Myxobolus ellipsoides*	Several round chromatic, deeply staining bodies, which are not observed after the spore membrane is formed (Figs. 41, 42).
13. Schroeder	*Sphæromyxum sabrazesi*	Chromatic, deeply staining bodies which are not observable when spore membrane is formed. (Comp. Figs. 30, 32 with Figs. 33, 34.)
14. Auerbach	*Henneguya psorospermica*	To judge after Figs. 6 to 18, extrusion of a chromatic body in cytoplasm. (Note, only sections to judge from.)
15. Georgevitch	*Henneguya gigantica*	Four deeply staining chromatic bodies called by the author degenerated nuclei.

spore, they disappear after the spore membrane is formed. It is proved that in *Chloromyxum leydigi* they are of glycogenous nature as well as the spore membrane itself and the polar threads. In some cases their number is irregular. These chromatic lumps may be products of nuclear division, but the true chromosomes have not been found. Those authors (Mercier, Awerinzew, Parisi) who believe they have shown a syncaryon forming, have also observed an extrusion of chromatin immediately after the union of the supposed micro- and macro-gametocyte. In Mercier's case a second diffusion of round bodies is shown *inside* the spore which corresponds with the facts observed in other species. *Ceratomyxa drepanopsettæ*

(Awerinzew) has only an extrusion of chromatin before the syncaryon formation, while in *Myxobolus ellipsoides* (Parisi) it occurs immediately after this phenomenon. These exceptions in the series, *i. e.*, that inside the spore no chromatin diffusion is observed, may be due in the case of Parisi to a limited number of studied forms and in the case of Awerinzew to the fact that the spore membrane in *Ceratomyxa* is formed very late. Yet these exceptions do not prevent the final statement that the darkly staining chromatic masses in the spore are not reduction nuclei, or restkernartige Gebilde, but play an important part in the development of the spore membrane.

The whole trend of our critical review leads up to the following conclusions:

1. Reduction in myxosporidia has thus far not been discovered.

2. The so-called reduction nuclei inside the spore are chromatic or glycogenous masses, which serve the spore-membrane formation.

3. The so-called residual nuclei of the disporoblastic forms can not be considered as identical with the somatic residual nuclei of the mono-, di- or poly-sporous non-disporoblastic forms. They are the functionless nuclei of the envelope cells of the disporoblastic forms.

4. The envelope cells can by their origin only be compared with those cells in the mono-, di- or polysporous nondisporoblastic forms which later give rise to the valve cells.

5. The somatic residual nuclei are well-defined in mono-, di- or poly-sporous nondisporoblastic myxosporidia. Their analogy has not thus far been discovered in disporoblastic polysporous forms.

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EVIDENCE FOR THE DEATH IN UTERO OF THE HOMOZYGOUS YELLOW MOUSE[1]

HEMAN L. IBSEN AND EMIL STEIGLEDER

CUENOT (1905) and Castle and Little (1910) have presented conclusive evidence that yellow mice are always heterozygous and hence cannot be made to breed true. Their combined results show that when yellows are mated together the proportion of yellows to non-yellows in the offspring is almost exactly 2:1 instead of the usual 3:1 ratio resulting from the mating of heterozygotes. Castle and Little seem justified on this account in assuming that the homozygous yellows are not viable, especially since the size of litter from the yellow × yellow mating is markedly smaller than that obtained from yellow × non-yellow or non-yellow × non-yellow matings.

Until quite recently no attempt had been made to determine embryologically the actual fate of the homozygous yellows. Since the present investigation was begun, however, Kirkham (1917) has published a preliminary statement of the results of such a study. His results, presented only in abstract, show that of 69 embryos from yellow × yellow parents, 26 or 37.8 per cent. were degenerating. For "non-yellows" he used albinos.[2] Of

[1] Papers from the Department of Experimental Breeding, Wisconsin Agricultural Experiment Station, No. 11. Published with the approval of the Director of the Station.

[The problem of the fate of the homozygous yellow mouse was undertaken at my suggestion during the summer of 1916 by Mr. Steigleder and the experimental work on which the present paper is based was done entirely by him. As he was, however, unable to complete the problem the accumulated material and records were turned over to Dr. Ibsen, who has checked all records with the preserved embryos and is alone responsible for the tabulation, interpretation and presentation of the results.—L. J. Cole.]

[2] This selection of albinos for "non-yellows" was unfortunate, since they apparently were not tested genetically, and hence may or may not have carried the factor for yellow. From the fact that the proportion of dead embryos was markedly different we may accept Kirkham's assump-

the 84 embryos from albino parents, only 2, or 2.3 per cent., were degenerating. This makes it seem quite probable that the homozygous yellow zygote develops for a time and then dies.

In our study no attempt has been made to investigate the very early stages, as was done by Kirkham, but a large number of embryos have been obtained from non-suckling females pregnant from 13 to 19 days.[3] In all 688 embryos have been examined. These have been obtained from (1) yellow females mated to yellow males, (2) yellow females mated to non-yellow males (chocolates), (3) non-yellow females (chocolates) mated to yellow males, and (4) non-yellow females mated to non-yellow males. In this last mating most of the parents were self blacks.

During the investigation two distinct types of dead embryos were encountered, (1) those in which development had ceased shortly after implantation, corresponding to those described by Kirkham, and (2) a few which seem to have developed normally till about the thirteenth day and then died, presumably because of overcrowding in the uterus. These latter were characterized by their dead, yellow appearance and smaller size as contrasted with the living pink color and larger size of the normal embryos. The first kind has been designated "dead embryos *A*" in the tables, while the second kind is classed as "dead embryos *B*." We are primarily concerned with "dead embryos *A*," and it is to be understood that reference is to this type unless specifically stated otherwise.[4] Similarly, by "living embryos" we mean those which

tion that they were really genetically non-yellows, though this brings the argument dangerously close to reasoning in a circle. In our work a number of albinos were mated both to yellows and to non-yellows, but since the genetic constitution of the albinos was not sufficiently established in most cases, the embryos from these matings are not included in our tabulations.

[3] The normal duration of gestation in the mouse is about 21 days, but it is often less.

[4] We are indebted to Dr. Alva Wilson for sectioning for us dead embryos from each of the four types of matings. Microscopical examination of these has verified our previous conclusions as to their character.

were obviously alive when the mother was killed. As the females were killed by chloroforming, all embryos were usually dead by the time they were examined. The following tables give the results obtained. Each of the first four tables represents one of the four types of matings described above. In a few instances there has been some uncertainty as to the exact stage of gestation at which the embryos were removed, but in such cases this has been determined approximately by means of weights of the living embryos.

TABLE I

YELLOW ♀ × YELLOW ♂

Stage of Gestation	No. of Litters	Living Embryos	Dead Embryos *A*	Dead Embryos *B*	Percentage Dead Embryos *A*	Average Size of Litter	
						Living Embryos	Total Embryos
13 days.....	5	33	14		29.8	6.6	9.4
14 days.....	6	50	9		15.3	8.3	9.8
15 days.....	4	26	10	1	27.0	6.5	9.3
16 days.....	7	42	13	2	22.8	6.0	8.1
17 days.....	5	25	4		13.8	5.0	5.8
18 days.....	5	27	13		32.5	5.4	8.0
19 days.....	1		4		100.0		4.0
Total.....	33	203	67	3	24.54	6.15	8.27

TABLE II

YELLOW ♀ × NON-YELLOW ♂

Stage of Gestation	No. of Litters	Living Embryos	Dead Embryos *A*	Dead Embryos *B*	Percentage Dead Embryos *A*	Average Size of Litter	
						Living Embryos	Total Embryos
13 days.....	5	34	12		26.1	6.8	9.2
14 days.....	6	56	1		1.8	9.3	9.5
15 days.....	4	35	4		10.3	8.8	9.8
16 days.....	2	14	8		36.4	7.0	11.0
17 days.....	3	25	1		3.8	8.3	8.7
18 days.....	3	17	1	1	5.3	5.7	6.3
19 days.....	1	10				10.0	10.0
Total	24	191	27	1	12.33	7.96	9.13

The data presented agree in the main with Kirkham's, but our percentage of dead embryos *A*, from the yellow × yellow mating (Table I) is considerably less than his, while our percentage from the non-yellow × non-yellow

mating (Table IV) is somewhat higher. Theoretically, if we assume that a certain proportion of embryos of other gametic composition die from unknown causes in mice of all colors and that all the homozygous yellows are

TABLE III

NON-YELLOW ♀ × YELLOW ♂

Stage of Gestation	No. of Litters	Living Embryos	Dead Embryos *A*	Dead Embryos *B*	Percentage Dead Embryos *A*	Average Size of Litter	
						Living Embryos	Total Embryos
13 days.....	1	2	1		33.3	2.0	3.0
14 days.....	1	6				6.0	6.0
15 days.....	2	15				7.5	7.5
16 days.....	2	20	1	1	4.5	10.0	11.0
17 days.....	2	11				5.5	5.5
18 days.....	1	8	1		11.0	8.0	9.0
19 days.....							
Total.....	9	62	3	1	4.55	6.89	7.33

TABLE IV

NON-YELLOW ♀ × NON-YELLOW ♂

Stage of Gestation	No. of Litters	Living Embryos	Dead Embryos *A*	Dead Embryos *B*	Percentage Dead Embryos *A*	Average Size of Litter	
						Living Embryos	Total Embryos
13 days.....	3	22	3		12.0	7.3	8.3
14 days.....	3	28	1		3.4	9.3	9.7
15 days.....							
16 days.....	3	22		2		7.3	8.0
17 days.....	3	19				6.3	6.3
18 days.....	3	26	3		10.3	8.7	9.7
19 days.....	1	4				4.0	4.0
Total.....	16	121	7	2	5.38	7.56	8.13

represented as dead embryos, then the difference between the percentages of dead embryos from the yellow × yellow mating and the non-yellow × non-yellow mating should be approximately 25 per cent. In our results the difference is 19.2 per cent., while in Kirkham's it is 35.5 per cent. Neither of these is especially close to the expected percentage. If, however, his results and ours are combined the difference is 23.0 per cent., which is close to expectation.

If instead of comparing yellow × yellow with non-yellow × non-yellow only, all matings other than yellow

× yellow are combined for this purpose, it is found that the dead embryos in these combined matings constitute 8.9 per cent. of all embryos present. Subtracting this from 24.5, the per cent. of dead embryos in the yellow × yellow mating, the difference is only 15.6 per cent. When all these results are combined with those of Kirkham the difference is still only 19.4 per cent., which is considerably lower than that obtained by using only the classes considered by him. An attempt will be made farther on to explain this deficiency.

Tables II and III, representing the reciprocal crosses of yellow × non-yellow, show marked contrasts in several respects. In the yellow ♀ × non-yellow ♂ mating (Table II) 12.3 per cent. of the embryos were dead, while in the non-yellow ♀ × yellow ♂ mating (Table III) the percentage is only 4.5 per cent. For both matings the percentage of dead embryos theoretically should be the same as in the non-yellow × non-yellow mating (Table IV), since in neither case is there the possibility of any of the offspring being homozygous yellows. The percentage in Table III is approximately the same as in Table IV, but in Table II it is much higher. It is well known that yellow females tend to take on more fat than females of other colors. There is a possibility that this physiological difference may also in some way influence the production of a greater number of dead embryos *A*. This is offered merely as a suggestion, since there is no direct evidence for or against it.

In discussing the stages of pregnancy in which the dead embryos are to be found, Kirkham makes a statement which does not accord with our observations. He says: "No degenerating embryos have been found in either white [albino] or yellow mice pregnant more than sixteen days." He believes complete resorption of the degenerating embryos has taken place by the end of the sixteenth day. Table V, which is a summary of all our four types of matings, indicates that in our material there is no marked decrease in the percentage of dead embryos toward the end of pregnancy.

TABLE V

RELATION OF DEAD EMBRYOS TO STAGE OF PREGNANCY FOR THE FOUR TYPES OF MATINGS

Stage of Gestation	Dead Embryos *A*	Total Embryos (Including *B*)	Percentage of Dead Embryos *A*
13 days	30	121	24.8
14 days	11	151	7.3
15 days	14	91	15.4
16 days	22	125	17.6
17 days	5	85	5.9
18 days	18	97	18.6
19 days	4	18	22.2
Total	104	688	15.12

As previously stated, Cuénot, and Castle and Little noted that the average number per litter is less from yellow females mated to yellow males than from any other type of mating. Our results, summarized in Table VI, bear this out. Here it will be noted that the average number of living embryos is less for mating 1 than for any

TABLE VI

LITTER SIZE FOR THE DIFFERENT TYPES OF MATINGS

Type of Mating	No. of Litters	Average Number of Embryos per Litter		
		Living Embryos	Dead Embryos *A* and *B*	Total
1. Yellow ♀ × yellow ♂	33	6.15	2.12	8.27
2. Yellow ♀ × non-yellow ♂	24	7.96	1.17	9.13
3. Yellow ♂ × non-yellow ♀	9	6.89	0.44	7.33
(2 and 3 combined)	33	7.67	0.97	8.64
4. Non-yellow ♀ × non-yellow ♂	16	7.56	0.57	8.13
(2, 3 and 4 combined)	49	7.36	0.84	8.47

of the other types of matings singly or combined. It is evident that the average number of living embryos per litter represents those which would in the regular course of events have been born alive. In the yellow × yellow mating the average is only 6.15,[5] while for the other matings combined it is 7.63. The average number when the dead embryos are also included should be approximately the same in both cases, and this proves to be true, being 8.27 for yellow × yellow and 8.47 for all other matings.

[5] Two of the litters in this mating were made up entirely of dead embryos *A* and therefore had no living embryos. So far as living embryos are concerned their size would be 0, and they have been included as such with the other litters produced by this mating.

During 1916–17, the period at which the embryological study was carried on, numerous living litters of mice were born in the laboratory. Miss Sarah V. Jones, who was also working with mice, has generously furnished data from her own breeding experiments, and these have been incorporated with ours. Some of the mice in her experiments were black-and-tans, but as Dunn (1916) has shown that these are a form of yellow and are also always heterozygous, they have been classified as yellows. Table VII shows the average size of litters for the various types of matings.

TABLE VII

AVERAGE SIZE OF LITTERS BORN DURING 1916–17

(Figures in parentheses indicate the number of litters.)

Type of Mating	Average Number of Mice per Litter	
Type of Mating	At Birth	At Time Color was Recorded
1. Yellow ♀ × yellow ♂	5.36(140)	4.55(121)
2. Yellow ♀ × non-yellow ♂	6.21(88)	5.63(71)
3. Yellow ♂ × non-yellow ♀	7.02(50)	5.95(46)
(2 and 3 combined)	6.51(138)	5.76(117)
4. Non-yellow ♀ × non-yellow ♂	6.88(42)	5.49(37)
(2, 3 and 4 combined)	6.59(180)	5.69(154)

It will be seen that there is a deficiency of litter size from the yellow × yellow mating here as in the embryological material. Theoretically the average size of litter from yellow parents should be 75 per cent. of that from any of the other matings. Both Cuénot, and Castle and Little, however, have found it to be above 80 per cent. Our results are in accord with these findings.[6] Table VIII shows the percentages found by the various investigators. In order to make them comparable with the others our results as given in the table are for the living litters only and do not include the data from the embryological investigations.

[6] Miss Durham (1911) found very little difference in average litter size between the offspring from yellows × yellows and the offspring from matings where at least one of the parents was a non-yellow. She says: "Only mice which lived long enough to have their colors determined are included in these averages." It seemed possible to us that she had not found any

TABLE VIII

PROPORTIONATE SIZE OF LITTERS FROM YELLOW × YELLOW AND YELLOW × NON-YELLOW MATINGS

(Figures in parentheses represent number of litters.)

Authority	Average Size of Litter		Ratio of "Yellow × Yellow" to "Yellow × Non-yellow"
	Yellow × Yellow	Yellow × Non-yellow	
Cuénot (1909)	3.38(50)	3.74(50)	90.37 %
Castle and Little (1910)	4.71(277)	5.57(325)	84.63 %
Ibsen and Steigleder, 1917	5.36(140)	6.51(138)	82.33 %

The most striking fact brought out in this table is that as the average size of the litters increases the ratio tends to decrease and therefore to approach 75 per cent. It would seem from this that if one could secure a race of mice having a high enough average per litter the theoretical percentage could be obtained. Using Table VIII as a basis for computation, such a race should average approximately 10 young per litter in the non-yellow × yellow mating and consequently about 7.5 per litter for the yellow × yellow mating. It is not probable that a race of this sort exists.

Various theories have been advanced to explain this unexpectedly large litter size in the yellow × yellow mating. Two suggested by Castle and Little will be considered here. Both take as their starting-point that "the perishing of a pure [homozygous] yellow zygote makes possible the development of a certain number of *other* fertilized eggs." The explanation follows: "Two ways may be suggested in which this might come about. First, more eggs may normally be liberated at an ovulation than there are young born subsequently. In that case, failure of some eggs to become attached to the uterus may make the chances greater that the remainder will become at-

difference because the size of litter had not been recorded at birth. Our records have been gone over and the litter size found at the time the colors were recorded. Some litters did not live long enough to be recorded in this manner and hence could not be included. By referring to Table VII one can see that our results do not at all agree with Miss Durham's. As a matter of fact, in our material the percentage relation is lower than when the litter size was computed at birth, being almost exactly 79 per cent. when "yellow × yellow" is compared with "non-yellow × yellow."

tached, or the perishing of some may make the chances greater that the rest will successfully complete their development. Or secondly, the production of a relatively small number of young at one birth may lead indirectly to more free ovulation subsequently, and so to the production of a large litter at a second birth.''

It will be seen in the first place that the above theories are based on false premises. It was not known at the time these theories were proposed that the homozygous yellow zygote does *not* perish in the sense of disintegrating and finally disappearing. It merely ceases to develop after a certain stage has been reached, and then remains more or less stationary till parturition. It might still be maintained that since these undeveloped zygotes take up very little room there would still be the possibility for the ''other'' zygotes to develop. In that case the average number of total embryos (including dead embryos *A* and *B*) should be greater in the yellow $\times$ yellow mating than in the non-yellow $\times$ yellow mating. Our data, presented in Table VI, indicate that this is not the fact. The other theory that ''the production of a relatively small number of young at one birth may lead indirectly to more free ovulation subsequently, and so to the production of a larger litter at a second birth'' still remains a possibility so far as our data are concerned.

There are several other possibilities which seem worthy of consideration. Instead of looking for causes that tend to *increase* the size of litters from yellows $\times$ yellows it may be profitable to determine if possible causes that may *decrease* the size of litters from the non-yellow $\times$ yellow mating. The first and most obvious possibility is that overcrowding in the uterus may have this effect by causing the death of some of the embryos. However, none of our results bear this out. In the first place there are proportionately just as many dead embryos *B* (whose death is probably caused by overcrowding) in the one type of mating as in the other, and in the second place this would mean that for our race of mice, having a high average litter size, there should be a proportionately

large death rate due to overcrowding, and this would tend to increase the percentage relation between "yellow × yellow" and the "non-yellow × yellow" instead of decreasing it, which actually is the case.

We know that dead embryos *B* do not materially decrease the litter size in the non-yellow × yellow mating, but on the other hand we have clear evidence that dead embryos *A* have a decided effect in this respect. The only manner we could postulate in which this could affect the litter-size percentage relation for the two contrasted types of matings would be to assume that in the yellow × yellow mating dead embryos *A* are due almost entirely to the fact of their being homozygous yellows, while in the non-yellow × yellow mating separate agencies are at work producing an appreciable number of deaths. If a more careful examination of dead embryos *A* should reveal rather easily distinguishable differences this explanation could be tested.

There is still another explanation which so far as we know has never been suggested, and which has more evidence in its favor than any of the others proposed. When yellows are mated to yellows it is to be expected that some of the litters, especially if they are small, will consist entirely of homozygous yellows. Since these do not complete development, the entire litter will be composed of dead embryos *A* and consequently there will be no living embryos born at parturition. Such a litter will therefore be of 0 size. In our embryological investigation two litters consisting entirely of dead embryos *A* were found in the yellow × yellow mating and none in the other types of matings. Even with these two litters of 0 size in the yellow × yellow mating the average size of litter as compared to the litters of the non-yellow × yellow mating was not sufficiently low to bring the percentage relation down to 75 per cent. It is, however, 80.18 per cent., which is appreciably lower than the percentage obtained from litters of animals allowed to give birth to their young. (See Table VIII.) In the latter case it would naturally be impossible to detect the litters of 0 size.

In our embryological study there were 33 litters in the yellow × yellow mating. Of these, as previously stated, two consisted entirely of dead embryos *A*. This means that for 31 litters containing living embryos there were two that did not, or 6.45 per cent. If we assume a like

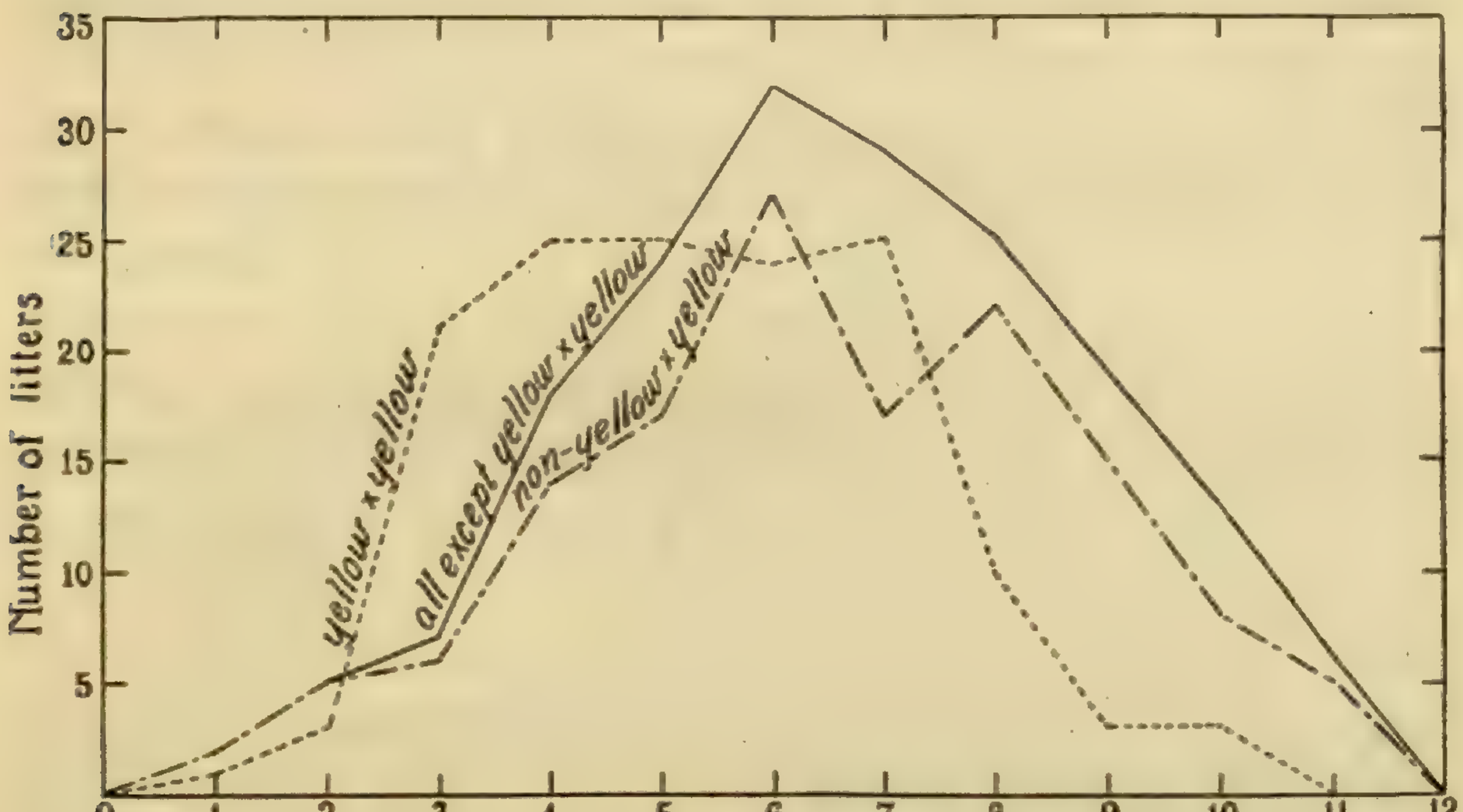

FIG. 1.

percentage of litters of 0 size with our 140 normally born litters from "yellow × yellow" (Table VIII) the total number of litters would be increased to 149 and their average size would be 5.04 instead of 5.36. Taking 5.04 as a basis for comparison with 6.51, the average size of litters from the non-yellow × yellow mating, we find that the percentage relation is 77.42 per cent. This is reasonably close to 75 per cent., the expected percentage. In this connection it may be well to call attention to a fact, previously stated, that as the average litter size increases the percentage relation tends to approach 75 per cent. This is exactly what one should expect. For with increased size of litter there would be fewer litters of 0 size, and the averages as actually found would more nearly represent the true averages.

Curves (Fig. 1) have been constructed showing the frequency of the litter sizes for certain of the types of matings. The data upon which they are based may be found in Table IX. A careful survey of these curves,

however, does not seem to lead to any very definite conclusions as to the special fitness of any of the various theories discussed in the preceding paragraphs. It will be seen that in the yellow × yellow mating there are proportionately more litters of small size and fewer of large size than are to be found in the other matings. This is what one should theoretically expect on all the theories.

TABLE IX

LITTERS BORN DURING 1916–17, CLASSIFIED ACCORDING TO NUMBER OF YOUNG IN LITTER

No. in Litter	Number of Litters			
	Yel. ♀ × Yel. ♂	Yel. ♀ × Non-yel. ♂	Yel. ♂ × Non-yel. ♀	Non.-yel. ♀ × Non.-yel. ♂
1	1	1	1	
2	3	3	2	
3	21	5	1	1
4	25	11	3	4
5	25	13	4	7
6	24	18	9	5
7	25	11	6	12
8	10	10	12	3
9	3	11	4	4
10	3	3	5	5
11		2	3	1
Total . . .	140	88	50	42

Summing up, we may say that all of our evidence tends to confirm the conclusion of Castle and Little that in mice homozygous yellow zygotes are produced in the yellow × yellow mating, but that these zygotes fail to develop normally after implantation in the uterus. Why this should be so is not evident and our investigation has not thrown any light on this point. It is possible a careful microscopical study of the embryos which die early might reveal some abnormality of development which would account for their failure to survive, but it is not probable that it would be of such a simple nature as the analogous cases of death of homozygous recessives lacking chlorophyll in corn and some other plants. It seems more probable that in mice there may be a "lethal factor," similar to those so well known in *Drosophila,* which is so closely linked to the factor for yellow that they are prac-

tically at the same locus and there is consequently no crossing over.

According to the investigations of Little (1915) an entirely similar condition obtains in regard to a dominant white spotting factor (*W*) in mice, which, like yellow, appears never to occur in a homozygous condition. Whether the homozygous individuals in this case also die at an early stage and might be found as dead embryos has not yet been determined. Little has, however, demonstrated (1917) that the two factors are independent in heredity, and that litters from yellows carrying *W*, mated *inter se*, average only three per litter (10 litters), while similar yellows mated to *ww* non-yellows have litters averaging 5 per litter (9 litters). Although the numbers are small the percentage relation for the two matings, 60 per cent., is quite close to the theoretical expectation, 56.3 per cent.

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SHORTER ARTICLES AND DISCUSSION

ON THE FAUNA OF GREAT SALT LAKE

In a recent number of the American Naturalist[1] appeared a paper entitled "Notes on the Fauna of Great Salt Lake," by Dr. Chas. T. Vorhies. From observations made by the present writer in the region at the mouth of Bear River, Utah, during the summer and fall months from 1914 to 1916 inclusive, information is available that supplements in part the data given by Dr. Vorhies.

Bear River, the largest of the three main tributaries of Great Salt Lake, breaks up into a series of channels at its mouth and forms a great delta at the northern end of Bear River Bay. Immediately below the mouth of the river the waters of the bay are freshened by the incoming river water. Conditions here vary greatly however from day to day, and at present heavy salt water frequently comes up as far as Slaughter Island at the lower part of the marsh area in the river delta. Below this point the surface water coming from the river may be fresh while at a depth of a few inches a stratum of brine may overlie the mud. On calm days this overlapping proceeds for long distances. The prevailing summer winds however are from the south and southwest and these drive the salt water in toward the marsh nearly every afternoon.

It is common belief that the Southern Pacific cut-off has served as a dam to separate Bear River Bay from the main lake (cf. Vorhies, p. 494). For a considerable distance this causeway is made up of trestle work allowing free interchange of water from either side. Although tests of the density of the water were not made, the writer is certain that the difference in content of salts between the water on the north and south sides of the cut-off is slight while water sufficiently saline to enable the life characteristic of the Lake to flourish is found at least twelve miles above the cut-off and within four or five miles of the point at which the main channel of Bear River opens into what is known as South Bay.

[1] Vol. LI, No. 608, August, 1917, pp. 494–499.

Brine shrimp, *Artemia fertilis* Verrill, occurred at this point in enormous numbers and adults and larvæ of alkali flies, *Ephydra gracilis* Packard and *E. hians* Say, were abundant. *E. subopaca* Loew was less common. The brine shrimp were gathered in great masses, and took advantage of the slightest depressions in the mud as shelters against the ever-fluctuating currents. Thousands frequently gathered in the lee of the boat during periods of observation by the writer.

In May and June adult *Ephydra* were found on mud, laid bare by water receding from the high spring levels, in which alkalis were rising through surface evaporation. On these areas the flies formed dense masses several feet square. The insects were busily probing or kneading the mud with their proboscides so that the surface was heavily pitted or stippled with small depressions that were visible at a distance of several feet. The greater part of these insects were *Ephydra gracilis*.

The statement made by Dr. Vorhies (p. 498) that "enemies play no part in keeping down the numbers of *Artemia*, or of *Ephydra* in the larval stage" is not corroborated by observations of the present writer. After the first of September each year shovelers, *Spatula clypeata* (Linnæus), began to congregate in the bay below the mouth of Bear River, and by October 1 thousands of these ducks were present. The birds lay in great banks on the open water, and it was not unusual to see such flocks that were at least two miles long and from one quarter to one half a mile broad. The shovelers were feeding almost entirely upon *Artemia fertilis* and larvæ and pupæ of *Ephydra*, and were crammed with them constantly. Usually this species of duck is not a good table bird but individuals shot here were all exceedingly fat, and the writer found them excellent eating. These ducks remained in fall until the fresh water bays were covered with ice. Another species of duck, the lesser scaup, *Marila affinis* (Eyton), came into this region from the north between October 2 and 12 each year, and by October 20, was abundant. These lesser scaups also frequented the lower bay, and, like the shovelers, fed to a large extent upon the brine shrimp and the immature stages of the alkali flies. At dusk on October 14, 1914, flocks of these ducks were observed from Promontory Point passing from Bear River Bay southwest past Fremont Island in the open lake. As there is no fresh water feeding ground in that direction it was assumed that they were going out

to feed at some favorable locality in the lake. Later in October the other ducks were joined by American goldeneyes, *Clangula c. americana* Bonaparte, while from observations it was certain that the green-winged teal, *Nettion carolinense* (Gmelin), at times fed upon this same food. The number of crustaceans and fly larvæ destroyed by these birds must be enormous.

In addition to these ducks great flocks containing thousands of Wilson's phalaropes, *Steganopus tricolor* Vieillot, and northern phalaropes, *Lobipes lobatus* (Linnæus), are found on the salt water during migrations, where these birds likewise feed upon the brine shrimp and the fly larvæ and pupæ. During October and November flocks of eared grebes, *Colymbus nigricollis californicus* (Heermann), were found on the lake along the cut-off where their food must have been taken from the same supply as none other suitable is found. It may be mentioned here that a considerable number of shovelers and many thousand eared grebes winter on Owen's Lake in California, where saline conditions are similar to those in Great Salt Lake, and where a similar fauna is found.

Avocets, *Recurvirostra americana* Gmelin, and black-necked stilts, *Himantopus mexicanus* (Müller), also fed upon *Artemia* and *Ephydra* at the mouth of Bear River, and no doubt these animals furnished food to other shore birds. Definite data on this point is not at hand, however, as all of the shore bird stomachs collected there have not yet been examined. It is probable that the ring-billed gull, *Larus delawarensis* Ord, and California gull, *Larus californicus* Lawrence, also take this same food at times.

From the facts outlined above it will be seen that the toll taken by birds from the brine shrimp and alkali fly larvæ and pupæ during the course of a season constitutes a mass of individuals almost beyond comprehension. The digestion of food by the birds concerned is always a rapid process, and with soft-bodied animals like the brine shrimp a considerable mass would be consumed each day; and the same is true of the larvæ and pupæ of the alkali flies. The immense number of these creatures in the waters of the lake must be attributed to the large number of offspring produced rather than to an absence of enemies.

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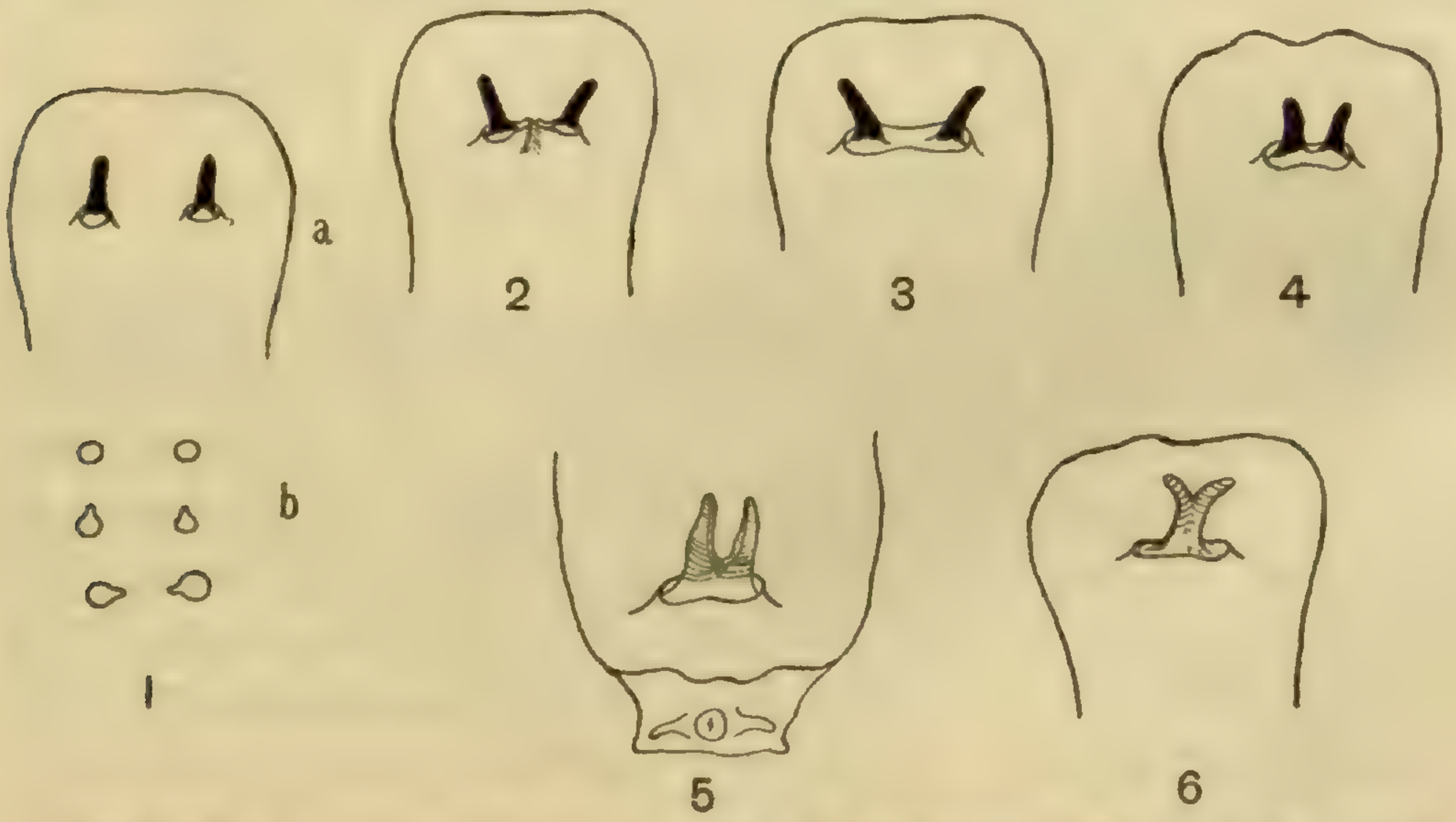
BIOLOGICAL SURVEY, WASHINGTON, D. C.

FUSION OF "RHINOPHORES" IN CHROMODORIS[1]

THERE have been found during the present spring nine specimens of the nudibranch *Chromodoris zebra* Heilprin which form a series exhibiting an interesting gradation in the degree of coalescence of the "rhinophores." The animals were each of average adult size, 10–12 cm. in length. In none of these cases was there any evidence that the structural variations had resulted from injury. In the period over which these individuals were obtained there were also collected about 1,000 normal specimens of the same species. These figures give, however, no precise idea of the relative frequency of "rhinophore" variation, because a larger number of specimens had been collected in previous years without any occurrence of these variations being observed.



FIGS. 1-6. Outlines of anterior ends of *Chromodoris zebra* Heilprin, showing increasing degrees of fusion of the "rhinophores." Fig. 5, frontal view; the rest, dorsal aspects. Fig. 1a, the normal condition; Fig. 1b, variation in the edges of the "rhinophoral" collars of three individuals.

The bases of the two "rhinophores" of *C. zebra* are, as in other Dorids, normally surrounded by well developed individual cylindrical collars. The distal termination of a collar is usually circular in outline, but occasionally pointed at one side (Fig. 1). In two specimens the "rhinophoral" collars were closely approximated, after the fashion outlined in Fig. 2. Three specimens were found in which the "rhinophoral" collars, and the depressions into which the "rhinophores" are separately retracted when stimulated, had completely fused (Fig. 3). In

[1] Contributions from the Bermuda Biological Station for Research, No. 75.

these animals the two "rhinophores" themselves were separated by their normal distance of about 1 cm. The next step in "rhinophore" fusion is illustrated in Fig. 4, one example having been collected. In another specimen the "rhinophores" were found to be closely united at the base (Fig. 5), while in the remaining two specimens that exhibit fusion of the "rhinophores" (Fig. 6) the process of coalescence had been pushed much further, a single stalk, giving rise at its free end to two short diverging projections, representing the normal pair of "rhinophores."

When a "rhinophore" of *C. zebra* is locally stimulated by being touched, it is retracted within its pocket, the basal collar usually contracting over it, while the companion "rhinophore" on the other side of the animal is usually not contracted. In other words, the "rhinophores" are, with reference to their retraction, subject to independent bilateral control. The process of retracting the "rhinophore" consists of two phases—the "rhinophore" is itself contractile, and it is in addition pulled down into its pocket by the action of muscles situated at its base. With the fused "rhinophores," even in such cases as that illustrated in Fig. 6, the independent bilateral control of the organs is preserved. If one tip be stimulated, that side of the compound "rhinophore" is contracted, the other (unless the stimulation be severe) remaining inert. Under slightly stronger stimulation applied to one tip of a compound "rhinophore," the contraction of the organ itself is immediately followed by the traction of muscles upon the same side of the base of the double "rhinophore," resulting in a bending of the whole structure toward the point of excitation.

The reactions of the abnormal specimens therefore support the view that these abnormal "rhinophores" have been produced by a process of fusion, probably resulting from the original close approximation of "rhinophoral" Anlagen. Two cases have been available for experiment in which one of the normally placed "rhinophores" possessed a divided tip; these divided-tip "rhinophores," superficially not unlike the single median structure above described, gave no evidence of independent control for the two tips, both parts contracting together when one tip was irritated.

It would appear that the development of the collar surrounding the base of the "rhinophore" is directly dependent upon the growth of the latter structure; in every case there was a close

correspondence between the bulk of the "rhinophores" and the dimensions of the collar or collars.

W. J. CROZIER

AGAR'S ISLAND, BERMUDA

NOTE ON THE HABITAT OF GEONEMERTES AGRICOLA[1]

THE terrestrial nemerteans include a small number of species, all belonging, apparently, to one genus, but widely scattered over the world. They occur conspicuously on islands, some of which are well removed from any large mainland. The origin of these land nemerteans is a matter of some interest, and several suggestions have been made relative to the manner of their evolution. One of these terrestrial nemerteans, *Geonemertes agricola* (W.-S.), was found at Bermuda by v. Willemoes-Suhm (1874). The anatomy of this species was subsequently described in detail by Coe (1904), who gave some attention, also, to the habits of the worm. These observers, as well as Verrill (1902), agree that *G. agricola* is to be found "only along the shores of mangrove swamps and on the adjacent hillsides" (Coe, p. 566). Coe found it "not only above high-water mark but also for some distance along a zone which is covered for a short time each day with sea water," but noted that the intertidal individuals were "as a rule smaller than those living in the soil which is a little above the reach of the tide, but in earth which is nearly saturated with salt water."

Standing bodies of fresh water are absent in Bermuda. Coe consequently held that this particular species, at least, represents a land nemertean which has almost certainly been derived directly from a marine ancestor, and not, as Montgomery (1895, p. 483) had argued for the generality of land nemerteans, from a fresh-water form.

During the past several years I have repeatedly encountered *G. agricola* in a type of habitat which is significantly different from that recorded for this nemertean by the observers just quoted. In the neighborhood of every large or small mangrove "creek" or swamp which I have examined, the worm has been found, in relatively considerable quantities, well below low-water mark even at spring tides. The species occurs in the localities

[1] Contributions from the Bermuda Biological Station for Research, No. 76.

listed by Coe, but is also common among the masses and sheets of matted sea weeds (*Laurentia, Valonia, Halimeda,* and associated plants) which cover the bottom of Fairyland Creek. Specimens were also obtained from under rocks situated a few feet beneath low-water level, in muddy bays bordered by mangroves, such as Tucker's Bay in Harrington Sound. The individuals collected in these places embraced white forms, some with a tinge of pink, others decidedly pink (as in the "pale" form figured by Coe, 1904, Pl. 1). They were 30–60 mm. in length, and some contained embryos.

In June and in July young *Geonemertes* were gotten among sea weeds in Fairyland Creek; these were 6–12 mm. in length. They were identified principally through the microscopic examination of the stylets and other organs. The stylets and stylet basis in these young specimens were of the juvenile type for this species, as figured by Coe (1904, Pl. 25, Figs. 21, 24, 25). These young specimens were in some cases pure white, in others tinged with "smoky brown." I found no pinkish specimens less than 30 mm. in length.

The observations upon the specimens of this species inhabiting salt water indicate, as Coe concluded from his study of the land-living individuals, that liberation of the young occurs in June and in July. My largest examples of *G. agricola* from the water were obtained in the spring.

Large specimens of *G. agricola* are negatively phototropic, the ocelli occupying the region of the body most sensitive toward light. They orient away from the light with diagrammatic precision. This response leads to their being found, during the day, under stones and about the roots of algæ.

It is hardly possible to credit the view that *G. agricola* has extended the variety of its habitats during the brief time since Coe's studies were made (1903); it is therefore necessary to believe that this species of nemertean is not only terrestrial in the proper sense, but truly marine as well. There seems no good ground upon which to distinguish and separate the individuals found respectively on land, in the intertidal zone, and definitely in the sea water. The terrestrial "variety" may then be regarded as having originated, perhaps not so very long ago, from the form which is undoubtedly marine—unless one is prepared to believe that, introduced as a terrestrial form, it has at some time secondarily taken to the sea after a protracted evo-

lution as a land animal. This case seems to have some resemblance to that of a grapsoid crab at Bermuda, *Sesarma ricordi* M.-Edw., of which a terrestrial variety has been described by Verrill (1908, p. 328). It is my impression that the larger marine specimens of *G. agricola* are less hardy, more easily caused to fragment by handling, than are those taken on land. This may, however, be merely a physiological consequence of differences in habitat, which could be exhibited within the life-history of a single individual. I have not been able to keep the salt water specimens alive after abruptly transferring them to damp earth. The young individuals, however, are quite hardy, and seem capable of enduring this treatment for several days at least.

These observations add further, and possibly final, weight to the argument that some, at least, of the land nemerteans have proceeded directly from ancestors inhabiting salt water.

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AGAR'S ISLAND, BERMUDA

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NOTES AND LITERATURE

SUNSPOTS, CLIMATIC FACTORS AND PLANT ACTIVITIES

VARIATIONS in solar radiation, if of sufficient magnitude, should be followed by variations in terrestrial climatic conditions. In the absence of long series of determinations of the heat radiated by the sun, meteorologists have turned to variation in the number of sunspots as a possible factor underlying variation in the climatic factors. This involves the assumption that a period of many sunspots differs from a period of few spots in heat and light radiation.

If such climatic factors as heat and precipitation be closely related to the number of sunspots, the number of sunspots should be a factor of importance in determining plant activities. In recent years attempts have been made to correlate growth, especially that recorded in the annual rings of trees, with number of sunspots.[1]

Since any attempt to relate growth phenomena to sunspot number presupposes relationships between climate and sunspot frequency, the botanist should be interested in the attempts of the meteorologist to ascertain the relationship between solar and terrestrial atmospheric phenomena. The purpose of this review is to call attention to certain recent discussions of this subject. For a review of earlier literature, the reader must refer to Hann's "Handbook of Climatology" and to the careful discussion from the biological side in Chapter XIX of Huntington's "Climatic Factor."[2]

Heretofore, those who have discussed the interdependence of terrestrial and solar phenomena have been content to plot curves for the two phenomena and to determine the existence of relationship between them by similar trends in the two curves.

The sources of error in such a method are very great. Furthermore, it gives no quantitative measure of intensity of relationship. Such a measure can only be secured by means of some correlation or contingency coefficient.

[1] Douglas, A. E., "A Method of Estimating Rainfall by the Growth of Trees." In Ellsworth Huntington's "The Climatic Factor," pp. 101–121.

[2] Huntington, E., "The Climatic Factor as Illustrated in Arid America," Pub. Carn. Inst. Wash., 192, 1914.

Walker,[3] the director general of observatories for India, has made a great advance in the investigation of the possible relationship between the number of sunspots and meteorological phenomena by applying the modern methods of correlation to the problem.

I have tabulated his three series of correlations for a large number of stations widely distributed over the earth, with the results given in the accompanying table.

Intensity of Correlation	Frequency of Correlations		
	Sunspots and Rainfall	Sunspots and Temperature	Sunspots and Pressure
−.59 to −.53	—	2	—
−.52 to −.46	1	1	2
−.45 to −.39	1	6	2
−.38 to −.32	6	7	3
−.31 to −.25	9	10	8
−.24 to −.18	17	12	8
−.17 to −.11	15	15	8
−.10 to −.04	21	14	15
−.03 to +.03	27	16	11
+.04 to +.10	13	4	8
+.11 to +.17	18	5	10
+.18 to +.24	9	4	8
+.25 to +.31	7	1	6
+.32 to +.38	6	—	2
+.39 to +.45	—	—	—
+.46 to +.52	1	—	—
Total number of stations.	151	97	91

Remembering that correlation is measured on a scale of −1 to +1, this table shows at once that there is no uniformity for the globe as a whole in the correlations between number of sunspots and either of the climatic factors considered. Instead coefficients for some stations are positive while those for others are negative. Thus as far as the data available go, they indicate that in some regions rainfall, temperature or barometric pressure are higher in periods of larger numbers of sunspots, whereas in other regions they are lower. The magnitude of the coefficients is generally low.[4] Over thirty per cent. of the constants

[3] Walker, Gilbert T., "Correlation in Seasonal Variation of Weather," IV–VI. Mem. Ind. Met. Dep., 21: 10–12, 17–118, 3 world maps, 1915.

[4] These correlations are based on such small samples that for their full interpretation the theory of the distribution of small correlations now being developed by "Student," Soper, Fisher, Young, Cave, Lee and Pearson must be considered. Nothing brought out by the work of these writers, which will be reviewed later, invalidates the general correctness of the conclusions reached here.

lie between + .10 and — .10. The probable errors of the constants are of about this magnitude.

The average values of the three sets of correlations are:

For sunspots and rainfall, 151 stations, $\bar{r} = -.0175$
For sunspots and temperature, 97 stations, $\bar{r} = -.1360$
For sunspots and pressure, 91 stations, $\bar{r} = -.0331$

While these averages are exceedingly small, all are negative in sign, indicating that for the globe as a whole lower rainfall, temperature and barometric pressure are associated with greater numbers of sunspots.

The same relationship is apparent if only the 76 stations for which all three relationships have been calculated be considered. The averages are:

For sunspots and rainfall, $\bar{r} = -.0349$
For sunspots and temperature, $\bar{r} = -.1534$
For sunspots and pressure, $\bar{r} = -.0486$

If in obtaining the average correlations the constants for the several stations are weighted with the number of years for which records are available the averages, indicated by the bars, are:

For sunspots and rainfall, 151 stations, $\bar{r} = -.0103$
For sunspots and temperature, 97 stations, $\bar{r} = -.1243$
For sunspots and pressure, 91 stations, $\bar{r} = -.0387$

Thus, however calculated, the averages indicate generally negative values of the correlation coefficient for all three relationships.

The same fact is brought out if the coefficients are classified according to sign only. Thus:

Number of Sunspots and:—	Frequency of Positive Correlation	Frequency of Zero Correlation	Frequency of Negative Correlation
Rainfall	70	1	80
Temperature	18	4	75
Pressure	38	3	50

For all three relationships the negative correlations are more numerous than those which are positive in sign.

In a brief review it is quite impossible to give in detail the meteorological considerations discussed in the original memoirs. Furthermore, the most of these do not directly concern the botanist. The conclusions of practical biological importance to be drawn from Walker's investigations seem to be the following:

a. The relationship between the number of sunspots and the annual record of terrestrial meteorological phenomena is very slender indeed. It is so slight that at the present time it is impossible to assert on the basis of the data of any one station alone that any relationship at all exists. Thus, as far as they go, these data hold out very little hope to the biologist of being able to correlate plant activities with sunspot number, unless light intensity be the means of solar influence.

b. For rainfall and barometric pressure the correlations are especially low. They average practically zero, but are apparently on the whole negative in sign.

c. The correlation between number of sunspots and terrestrial temperature is the most consistent and substantial of the three. The coefficients average about — .14. Thus years of larger numbers of sunspots are in the long run years of lower, *not higher*, terrestrial temperature.[5]

These results are directly opposed to the theories which seem to have prevailed among many writers.

J. ARTHUR HARRIS

[5] Possibly, as Walker suggests, superheating in equatorial regions may raise the temperature in the upper air but lower that at ground level. The temperatures in which the botanist is primarily interested are, however, those which may influence the film of vegetation which covers the globe.

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